

REVIEW

The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT)

R. VALENTA, J. LIDHOLM*, V. NIEDERBERGER†, B. HAYEK, D. KRAFT and H. GRÖNLUND*

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

**Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden and †Department of Otorhinolaryngology, Vienna General Hospital, University of Vienna, Vienna, Austria*

Summary

Current forms of allergy diagnosis and specific immunotherapy are performed with allergen extracts. Allergen extracts contain a variety of allergenic and nonallergenic components and their allergen content is often cumbersome to standardize. If used for diagnostic purposes, positive reactions to a given allergen extract will thus provide the information that an allergic subject is sensitized against extract components, but without identifying them. Likewise, extract-based immunotherapy cannot be adapted to the individual patient's sensitization pattern. Here we review progress in the field of molecular allergen characterization by recombinant DNA technology, leading to novel forms of component-resolved diagnostics (CRD) and immunotherapy (CRIT) based on recombinant allergens. Several studies have demonstrated advantages of recombinant allergen-based diagnosis. Using recombinant allergens in *in vitro* diagnostic devices, a patient's individual IgE reactivity profile can be quantitatively established. The presence of IgE to cross-reactive allergen components can thus be determined and used to predict clinically relevant sensitization to allergen sources which contain immunologically related allergens. Moreover it has been demonstrated that cocktails of recombinant allergens, matching the IgE epitope complexity present in natural allergen extracts, can be assembled. Component-resolved diagnosis will thus allow the precise selection of those molecules for specific immunotherapy to which a patient is actually sensitized. Recently, several recombinant hypoallergenic allergen derivatives have been developed for immunotherapy. The progress in allergen research achieved by the use of recombinant DNA technology holds promise that component-resolved diagnosis and immunotherapy may help refine the procedures of

allergy diagnosis and immunotherapy in the coming decade and beyond.

Introduction

The term atopy describes the genetically determined tendency of a given individual to mount IgE antibodies against otherwise harmless antigens (i.e. allergens) [1]. Almost 20% of the western world population suffer from Type I allergy which can therefore be considered a major health threat in industrialized countries [2]. While IgE constitutes the least abundant class of immunoglobulins [3,4], the interaction between these antibodies and allergens acts to elicit specific and powerful cellular reactions. Immediate symptoms result from cross-linking of effector cell (e.g. mast cell, basophil) -bound IgE antibodies by allergens and the subsequent release of inflammatory mediators such as histamine and leucotrienes [5]. Chronic manifestations of atopy involve activation of eosinophils [6] and allergen-specific T cells, the latter process being enhanced when allergens are presented via IgE that is bound to antigen-presenting cells via the high-affinity FcεRI [7].

Diagnosis of Type I allergy is based on the detection of allergen-specific IgE antibodies or the elicitation of immediate symptoms by provocation testing (e.g. skin testing, nasal, oral or bronchial provocation). While the symptoms of Type I allergy can be reduced by pharmacological treatment, allergen-specific immunotherapy probably represents the only causative allergy treatment [8]. Allergen-specific immunotherapy is based on the administration of increasing doses of allergens to induce a state of allergen-specific non-responsiveness [9]. Diagnosis as well as specific immunotherapy are currently performed with extracts obtained from natural allergen sources. Attempts to isolate and purify the disease-eliciting allergens from the natural allergen sources for diagnostic and therapeutic purposes have proven too difficult and expensive for routine application. However, with the introduction of recombinant DNA

Correspondence: R. Valenta, Molecular Immunopathology Group, Department of General and Experimental Pathology, Vienna General Hospital, University of Vienna, Austria.

technology in the field of allergen characterization, an increasing number of recombinant allergens with immunological properties comparable with the natural allergens have become available [10,11]. Based on the progress made in the field of recombinant allergens, this review introduces a new diagnostic and therapeutic concept for Type I allergy. Component-resolved diagnosis uses defined recombinant allergens to dissect the individual patient's IgE reactivity profile with the aim of identifying the disease-eliciting molecules. These can be genetically engineered or serve as templates for the design of synthetic hypoallergenic allergen derivatives with reduced anaphylactic activity for use in patient-tailored treatment (component-resolved immunotherapy).

Allergen sources, allergen extracts and allergens

The term allergen source defines the biological origin of allergens whereas an allergen extract represents a mixture of allergenic and nonallergenic moieties obtained by aqueous extraction of the allergen source. It is common, however, that the word 'allergen' is used in the meaning of allergen source or allergen extract. In our opinion, this is unfortunate and may cause confusion since 'allergen', at least in scientific communication, is defined as a molecular entity with the capacity to bind IgE antibodies and elicit allergic reactions. Thus, we would advocate a more restrictive and stringent use of the word 'allergen'. Figure 1 is intended to illustrate these definitions. Grass pollen (Fig. 1a: e.g. timothy grass, *Phleum pratense*) represents a potent and widely spread allergen source world-wide [12]. Up to 40% of all allergic individuals carry serum IgE antibodies reacting with grass pollen allergens and exhibit immediate-type symptoms upon contact with grass pollen [13]. Timothy grass pollen extract (Fig. 1b: lane PPE) contains a variety of allergenic and nonallergenic proteins and glycoproteins as well as other components such as carbohydrates, nucleic acids and various small metabolites. However, only certain timothy grass pollen proteins, represented at least in part by the purified recombinant and natural allergens shown in Fig. 1(b) (lanes r1–r12), bind IgE antibodies and elicit allergic reactions. Pure allergens can be obtained from the natural allergen sources (e.g. nPhl p 4), or by recombinant DNA technology through the introduction of allergen-encoding DNA into a foreign host organism [10,11]. Depending on the host organism, the recombinant allergens are expressed without (bacteria) or with (eukaryotic cells) post-translational modifications. When the expression of the allergen-encoding DNA is controlled by a strong promoter [14], the recombinant allergen can account for up to 50% of total cellular protein which greatly facilitates its subsequent purification. Alternatively, affinity-tagged recombinant allergens can be expressed and purified by a single step of

chromatography [15]. Recombinant allergens can be produced identically time and time again without genetic, biological or batch-to-batch variation of the protein. This ensures that allergen reagents of consistent quality and superior standardization can be obtained.

Although the distinction between the terms 'allergen source', 'allergen extract' and 'allergen' may seem trivial, it is important for the understanding of different aspects of the allergic disease. For example, it is known that allergic individuals may show inconsistent allergic reactions upon contact with an allergen source at different, seemingly comparable occasions. This may happen when the reaction is due to an allergen that occurs as a sporadic contamination in an unrelated allergen source (e.g. presence of mite allergens in animal hair dander extracts) [16], when certain allergens are, for developmental or other reasons, not expressed in the allergen source (e.g. many pollen allergens are expressed only in mature pollen [17]; the expression of mould allergens varies greatly depending on culture conditions [18]) or when symptom-eliciting allergens are poorly extracted from the allergen source or become degraded (e.g. plant food allergens [19]). In contrast, the use of defined allergens in diagnostic tests will enable unambiguous, specificity-resolved analysis of the patient's IgE reactivity profile in a way which is not possible with conventional, extract-based technology. This may also be of interest in the context of monitoring immunological changes occurring naturally or following immunotherapy.

Many allergic individuals exhibit allergic symptoms after contact with various unrelated allergen sources. For example, birch pollen-allergic patients frequently experience oral allergic reactions after eating certain fruits, vegetables or spices, a phenomenon which may be due to the presence of structurally and immunologically related cross-reactive allergens in the different sources [20,21], although they may also represent genuine responses against unique epitopes in each of the sources. All of the examples given above emphasize that diagnosis using allergen extracts can serve to identify sensitization to a certain allergen source but will give no information regarding the molecular identity of the disease-eliciting allergen and the immunological mechanisms operative. In contrast, using recombinant allergens it is possible to measure humoral, cellular and biological responses to defined molecules. For the concept of using defined allergens (molecules), as opposed to allergen extracts (mixtures of allergens and other nonallergenic components) for diagnosis of Type I allergy, we propose the term component-resolved diagnosis.

The concept of component-resolved diagnostics

Molecular biological techniques were initially applied to reveal the primary structure and molecular identity of

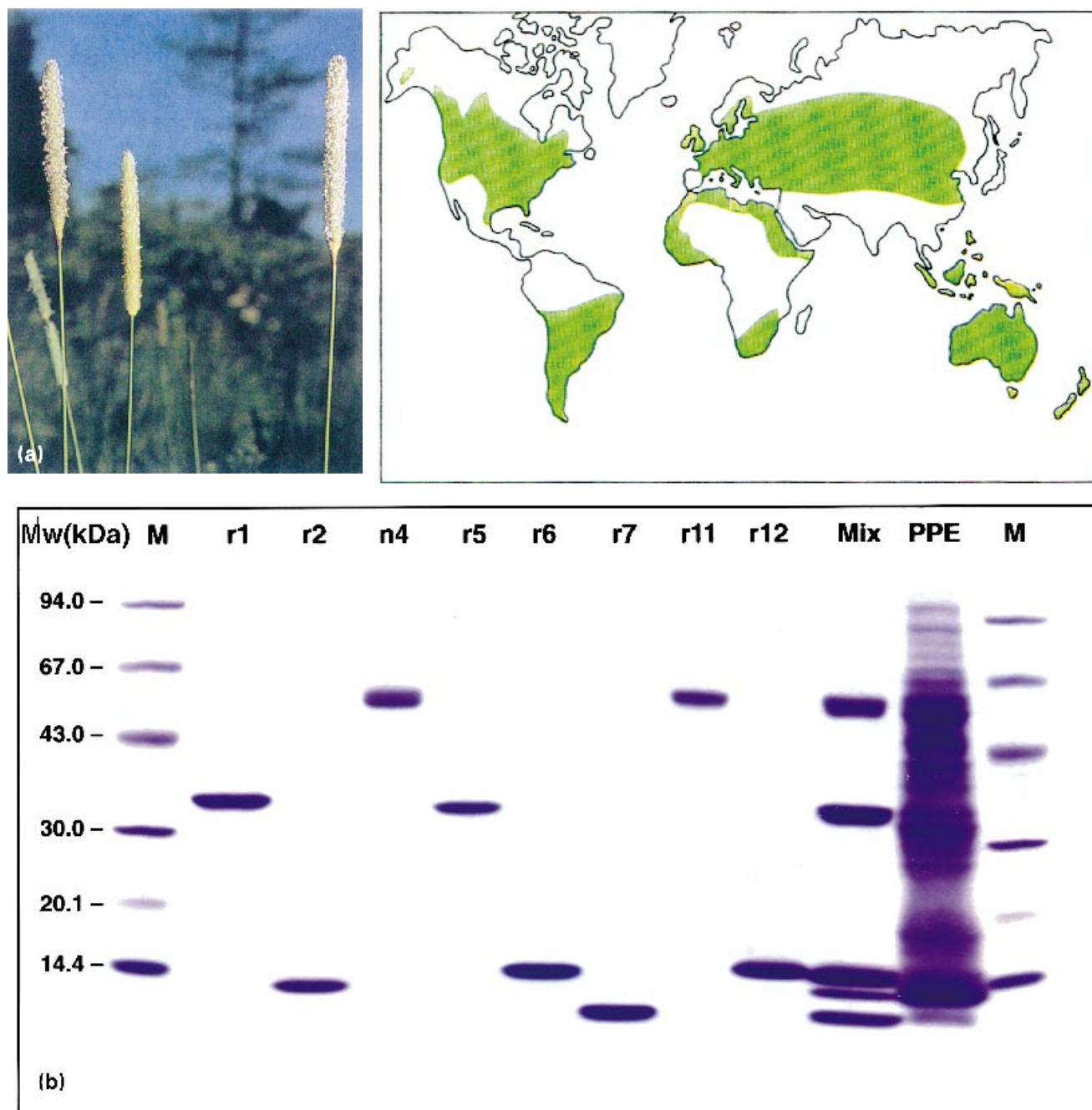


Fig. 1. Timothy grass represents one of the most potent allergen sources world-wide (Fig. 1a: reproduced from Allergy-Which allergens? Pharmacia AB, Uppsala, Sweden). Timothy grass pollen (*Phleum pratense*) extract (Fig. 1b: PPE) contains a great variety of allergenic and nonallergenic proteins. Purified recombinant (Fig. 1b: r1: rPhl p 1; r2: rPhl p 2; r5: rPhl p 5; r6: rPhl p 6; r7: rPhl p 7; r11: rPhl p 11; r12: rPhl p 12) and natural (Fig. 1b: n4: natural Phl p 4) timothy grass pollen allergens can be produced and used separately or in controlled proportion combination (Fig. 1b: mix), as desired. Molecular weights are indicated by a molecular weight standard.

allergens rather than to produce recombinant allergens for clinical applications [10]. The idea that it may be possible to produce panels of recombinant allergens for diagnostic and therapeutic purposes, which may complement or challenge

the use of natural allergen extracts has however, drawn support from several findings. Firstly, sequence analysis of allergens indicated that structurally related allergens exist not only in more closely related allergen sources, but also in

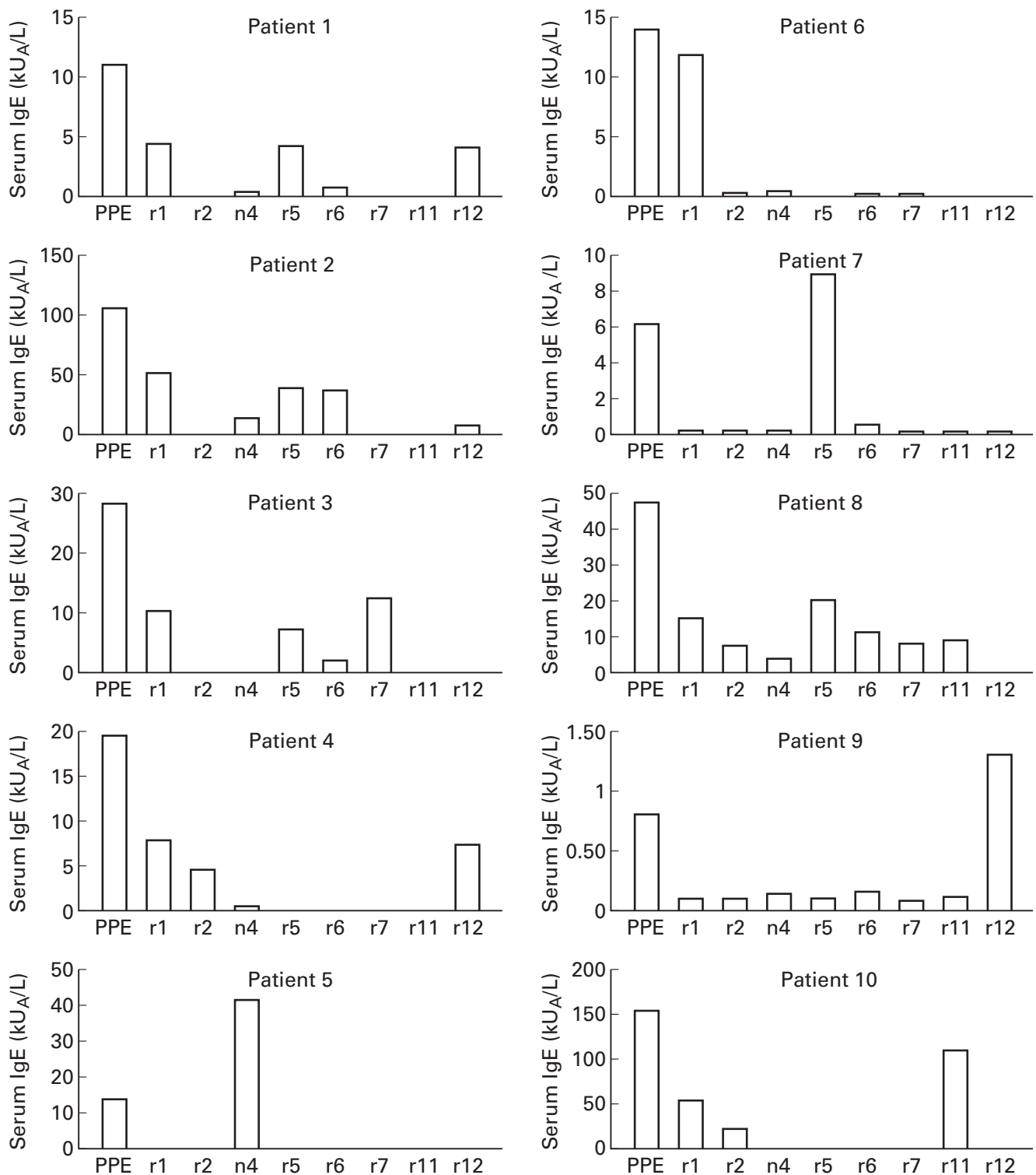


Fig. 2. Component-resolved diagnosis of grass pollen allergy. Quantitative IgE reactivity profiles were established for 10 grass pollen-allergic patients (1–10), showing their individual sensitization pattern. Serum IgE levels to timothy grass pollen extract (PPE), and purified grass pollen allergens (r1, r2, n4, r5, r6, r7, r11, r12) were determined using the Pharmacia CAP System. r1 = rPhl p 1, r2 = rPhl p 2, n1 = nPhl p 4, r5 = rPhl p 5, r6 = rPhl p 6, r7 = rPhl p 7, r11 = rPhl p 11 and r12 = rPhl p 12

taxonomically distant species, and that these cross-reactive molecules are often not strictly discriminated by the patient's immune system [22]. Secondly, immunological and biological investigations have shown that many recombinant allergens behave similar to their natural counterparts [11]. Thirdly, recombinant allergens which display most of the IgE epitopes present in natural allergen extracts could be produced and finally, a limited number of recombinant allergens were found to be sufficient to diagnose most patients with tree and grass pollen allergy [23–25]. As exemplified in Fig. 5, it is possible in most cases (99%, $n = 150$) to diagnose allergic sensitization to timothy grass pollen by IgE serology using a limited panel of defined allergens derived from this source. Moreover, the individual patient's sensitization profile can be precisely determined with the single allergens, thus allowing component-resolved diagnosis. CRD simultaneously allows determination of the frequency of IgE recognition of the individual timothy grass pollen allergens and measurement of the amount of grass pollen-specific IgE antibodies directed against each of the components [26]. CRD clearly demonstrates that grass pollen-allergic patients differ widely with respect to their IgE reactivity profiles. CRD also makes it possible to measure changes of allergen-specific IgE levels following natural allergen exposure, pharmacotherapy and specific immunotherapy, which may be of particular interest in relation to disease progression or treatment [27].

IgE recognition of cross-reactive recombinant allergens: a predictor for broad clinical sensitization?

It may be argued that as long as appropriate forms of component-resolved immunotherapy are not available, CRD will provide little advantage over conventional extract-based diagnosis and only serve to make allergy diagnosis more complex. By contrast, we believe that the application of CRD will generate valuable information relating to the clinical significance of allergens, eventually leading to the design of diagnostic tests for more precise clinical questions or with enhanced diagnostic or prognostic value. A positive reaction to an allergen with cross-reactive potential may predict allergic reactions to all those allergen sources which contain immunologically related molecules. For example, two EF-hand calcium-binding proteins have been identified as cross-reactive allergens in pollens of trees [28–32], grasses [33–35] and weeds [36]. Serum IgE antibodies from a timothy grass pollen-allergic patient, binding to the two EF-hand allergen from timothy grass pollen, Phl p 7, are likely to cross-react with pollen proteins from most plants, in particular with other grass species (including corn), trees of the Fagales order, olive and weeds (Fig. 3). More importantly, the cross-reactivity of IgE antibodies can be shown to produce a skin test

reaction to an EF-hand allergen from an unrelated plant species (Fig. 4). Likewise, IgE reactivity to other highly cross-reactive allergens such as profilin [37–41], animal albumin [42] and birch pollen allergen Bet v 1 [20,43,44] may be used to interpret clinical allergy symptoms and to predict reactions to materials containing related allergens.

Will recombinant allergen mixtures provide similar results as natural allergen extracts?

Allergen extracts contain many different proteins of allergenic potential and each of those may exist as multiple variants differing slightly in their amino acid sequence or post-translational modifications [45]. Moreover, there is evidence that certain recombinant allergens, when produced in prokaryotic expression systems, fail to behave comparably with their natural counterparts [46]. The question therefore arises whether it will be possible to replace natural allergen extracts by combinations of recombinant allergens without losing sensitivity in diagnostic tests. For tree and grass pollen allergy it was clearly demonstrated that mixtures of a few recombinant allergens can reproduce the allergenic complexity of natural allergen extracts required for *in vitro* allergy diagnosis [23,24] and that these allergens contained most of the IgE epitopes present in the corresponding natural pollen extracts [47,48]. Figure 5 shows the correlation of IgE determinations obtained using natural timothy grass pollen extract and a mixture of defined timothy grass pollen allergens. Out of all 150 patient samples tested, 148 (99%) exhibited comparable IgE reactivity with the natural extract and the mixture of purified

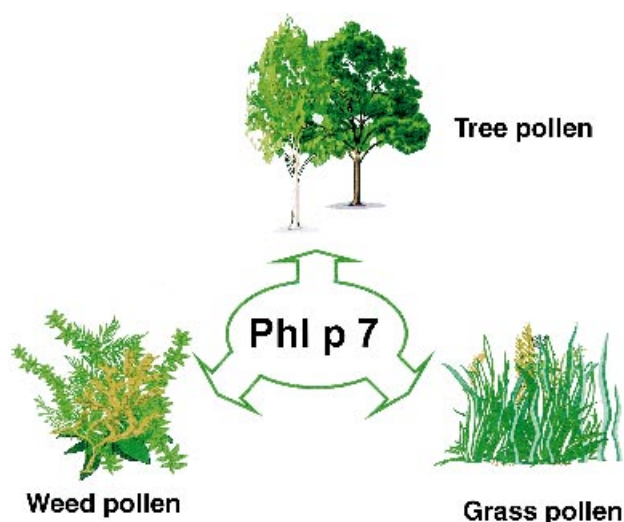


Fig. 3. Reactivity to two EF-hand calcium-binding pollen allergens (e.g. Phl p 7) predicts clinical sensitization to pollens of unrelated plant species.



Fig. 4. Aln g 4 and Phl p 7, the two EF-hand allergens from alder and timothy grass pollen induce immediate type skin reactions in sensitized patients. Two allergic patients carrying Phl p 7-specific serum IgE antibodies (A, B) and a nonatopic individual were skin prick tested with various concentrations (0.1–40 $\mu\text{g}/\text{mL}$) of purified rAln g 4 (Fig. 4a) or rPhl p 7 (Fig. 4b). In addition, individuals were tested with histamine (+ or H), sodium chloride solution (–), timothy grass (P or Phl) or birch pollen extract (B). The outer boundary of each weal area was marked with a ball point pen.

allergens, suggesting that it should be feasible to replace even complex allergen extracts by defined combinations of recombinant allergen reagents without losing sensitivity in the screening tests.

The concept of component-resolved immunotherapy

Specific immunotherapy is based on the administration of increasing doses of the disease-eliciting allergens to induce a state of non-responsiveness [8,9]. Since the introduction of immunotherapy by Noon in 1911 [49], the treatment has been modified to reduce the frequency of anaphylactic side-effects. These modifications include adsorption of the allergen extracts to suitable carriers (adjuvants) to prevent systemic reactions, attenuation of the allergenic activity of the extracts by denaturation (allergoids) [50], and different modes of administration. Although progress has been made regarding the standardization of allergen extracts by using

monoclonal antibodies, current forms of extract-based treatment cannot be patient-tailored to include relevant allergens only. Rather, all patients showing allergic reactions to proteins present in a particular allergen source are treated with the same complex extract regardless of their individual sensitization profiles. The allergen extract is commonly administered with an adjuvant and may therefore induce immune reactions against extract components not recognized before treatment, and such immunotherapy-induced sensitization has indeed been reported to grass pollen allergens [27,51].

CRD allows the individual patient's sensitization profile to be determined and the selection of exactly those allergens for immunotherapy to which the patient carries specific IgE. Immunotherapy can then be performed with no other proteins than those required to address the individual patient's sensitization problem component-resolved immunotherapy (CRIT). The use of purified recombinant allergens will allow the production of adsorbates for immunotherapy

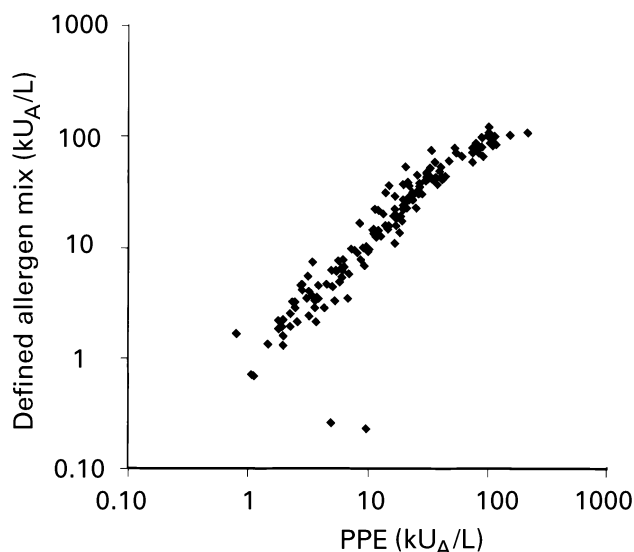


Fig. 5. A mixture of purified recombinant and natural timothy grass pollen allergens can be shown to reproduce virtually the full IgE binding capacity of a natural timothy grass pollen extract required for *in vitro* diagnosis of grass pollen allergy. Specific serum IgE levels (150 sera) to the defined allergen mixture are shown on the y-axis and specific IgE levels to timothy grass pollen extract (PPE) were plotted on the x-axis.

which contain defined amounts of purified allergens and hence meet the standards of regular vaccines [8]. In this context it has been demonstrated that immunization of mice with purified recombinant allergens induced IgG antibodies which reacted with the natural counterparts, bound to epitopes recognized by human IgE and blocked human IgE binding to the allergens as well as allergen-induced histamine release from basophils [52]. Moreover it is possible to genetically engineer recombinant allergen derivatives with attenuated allergenic activity. Several strategies have been successfully used to generate hypoallergenic allergen derivatives, including the production of allergen fragments which either bear only single IgE binding sites (IgE-binding haptens) [53] or fragments which lack or have greatly reduced IgE-binding capacity [54,55]. Naturally occurring isoforms of reduced allergenicity have been reported [56] and allergen mutants with reduced IgE binding capacity have been engineered [57–62]. Further, recombinant production of birch pollen allergen Bet v 1 as oligomers has been found to result in hypoallergenic properties of the protein [63]. Clinical evaluation of the allergenic activity of rBet v 1 fragments and oligomers has demonstrated that most patients tolerated at least a 100-fold higher dose of the hypoallergenic derivatives as compared with wild-type rBet v 1 [64,65]. It is expected that immunization with high doses of recombinant hypoallergenic derivatives will preferentially induce a TH1-like immune response which

would be accompanied by induction of blocking IgG antibodies [66–68]. Immunotherapy studies will now be required to establish whether CRIT performed with hypoallergenic recombinant allergens is effective and safe.

Acknowledgements

This study was supported by grant Y78GEN of the Austrian Science Fund, by the ICP project of the Austrian Ministry of Science and Transports and by a grant from Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden.

References

- 1 Kay AB. Allergy and Allergic Diseases. Oxford: Blackwell Science 1997.
- 2 Casolaro S, Georas SN, Song Z, Ono SJ. Biology and genetics of atopic disease. *Curr Opin Immunol* 1996; 8:796–803.
- 3 Ishizaka K, Ishizaka T, Hornbrook MM. Physicochemical properties of human reaginic antibody. *J Immunol* 1966; 97:840–5.
- 4 Johansson SGO, Bennich H. Immunological studies of an atypical (myeloma) immunoglobulin. *Immunology* 1967; 13:381–94.
- 5 Segal DM, Taurog JD, Metzger H. Dimeric immunoglobulin E serves as a unit signal for mast cell degranulation. *Proc Natl Acad Sci USA* 1977; 41:457–67.
- 6 Kay AB, Barata L, Meng Q, Durham SR, Ying S. Eosinophils and eosinophil-associated cytokines in allergic inflammation. *Int Arch Allergy Immunol* 1997; 113:196–9.
- 7 Stingl G, Maurer D. IgE-mediated allergen presentation via Fc epsilon RI on antigen-presenting cells. *Int Arch Allergy Immunol* 1997; 113:24–9.
- 8 Bousquet J, Lockey R, Malling HJ and the WHO panel members. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998; 102:558–62.
- 9 Durham SR, Till SJ. Immunological changes associated with allergen immunotherapy. *J Allergy Clin Immunol* 1998; 102:157–64.
- 10 Valenta R, Kraft D. Recombinant allergens for diagnosis and therapy of allergic diseases. *Curr Opin Immunol* 1995; 7:751–6.
- 11 Valenta R, Vrtala S, Laffer S, Spitzauer S, Kraft D. Recombinant allergens. *Allergy* 1998; 53:552–61.
- 12 Lockey RF, Bukantz SC, eds. Allergen immunotherapy, 2nd edn. New York, NY: Marcel Dekker, 1998.
- 13 Freidhoff LR, Ehrlich-Kautzky E, Grant JH, Meyers DA, Marsh DG. A study of the human immune response to *Lolium perenne* (rye) pollen and its components, Lol p 1 and Lol p 2 (rye 1 and rye 2). *J Allergy Clin Immunol* 1986; 78:1190–201.
- 14 Studier FW, Rosenberg AH, Dunn JJ, Dubendorff JW. Use of T7 RNA polymerase to direct expression of cloned genes. *Meth Enzymol* 1990; 185:60–89.
- 15 Van Dyke MW, Sirito M, Sawadogo M. Single-step purification of bacterially expressed polypeptides containing an oligohistidine domain. *Gene* 1992; 111:99–104.

- 16 van der Veen Mj, Mulder M, Witteman AM et al. False-positive skin prick test responses to commercially available dog dander extracts caused by contamination with house dust mite (*Dermatophagoides pteronyssinus*) allergens. *J Allergy Clin Immunol* 1996; 98:1028–34.
- 17 Mittermann I, Swoboda I, Pierson E et al. Molecular cloning and characterization of profilin from tobacco (*Nicotiana tabacum*): Increased profilin expression during pollen maturation. *Plant Mol Biol* 1995; 27:137–46.
- 18 Cramer R. Recombinant *Aspergillus fumigatus* allergens: from the nucleotide sequences to clinical applications. *Int Arch Allergy Immunol* 1998; 115:99–114.
- 19 Bousquet J, Valenta R. In vivo and in vitro use of recombinant allergens. *ACI Intern* 1994; 6:54–9.
- 20 Ebner C, Hirschwehr R, Bauer L et al. 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; 95:962–9.
- 21 Valenta R, Kraft D. Type I allergic reactions to plant-derived food: a consequence of primary sensitization to pollen allergens. *J Allergy Clin Immunol* 1996; 97:893–5.
- 22 Valenta R, Steinberger P, Duchêne M, Kraft D. Immunological and structural similarities among allergens: Prerequisite for a specific and component-based therapy of allergy. *Immunol Cell Biol* 1996; 74:187–94.
- 23 Valenta R, Duchêne M, Vrtala S et al. Recombinant allergens for immunoblot diagnosis of tree-pollen allergy. *J Allergy Clin Immunol* 1991; 88:889–94.
- 24 Valenta R, Vrtala S, Ebner C, Kraft D, Scheiner O. Diagnosis of grass pollen allergy with recombinant timothy grass (*Phleum pratense*) pollen allergens. *Int Arch Allergy Immunol* 1992; 97:287–94.
- 25 Laffer S, Spitzauer S, Susani M et al. Comparison of recombinant timothy grass pollen allergens with natural extract for diagnosis of grass pollen allergy in different populations. *J Allergy Clin Immunol* 1996; 98:625–58.
- 26 Laffer S, Vrtala S, Duchêne M et al. IgE binding capacity of recombinant timothy grass (*Phleum pratense*) pollen allergens. *J Allergy Clin Immunol* 1994; 94:88–94.
- 27 Ball T, Sperr WR, Valent P et al. Induction of antibody responses to new B cell epitopes indicates vaccination character of allergen immunotherapy. *Eur J Immunol* 1999, in press.
- 28 Twardosz A, Hayek B, Seiberler S et al. Molecular characterization, expression in *Escherichia coli*, and epitope analysis of a two EF-hand calcium-binding birch pollen allergen, Bet v 4. *Biochem Biophys Res Comm* 1997; 239:197–204.
- 29 Engel E, Richter K, Obermeyer G et al. Immunological and biological properties of Bet v 4, a novel birch pollen allergen with two EF-hand calcium-binding domains. *J Biol Chem* 1997; 272:28630–7.
- 30 Hayek B, Vangelista L, Pastore A et al. Molecular and immunologic characterization of a highly cross-reactive two EF-hand calcium-binding alder pollen allergen, Aln g 4: Structural basis for calcium-modulated IgE recognition. *J Immunol* 1998; 161:7031–9.
- 31 Batanero E, Villalba M, Ledesma A, Puente XS, Rodriguez R. Ole e 3, an olive-tree allergen, belongs to a widespread family of pollen proteins. *Eur J Biochem* 1996; 241:772–8.
- 32 Ledesma A, Villalba M, Batanero E, Rodriguez R. Molecular cloning and expression of active Ole e 3, a major allergen from olive tree pollen and member of a novel family of Ca²⁺-binding proteins (polcalcins) involved in allergy. *Eur J Biochem* 1998; 258:454–9.
- 33 Suphioglu C, Ferreira F, Knox RB. Molecular cloning and immunological characterization of Cyn d 7, a novel calcium-binding allergen from Bermuda grass pollen. *FEBS Lett* 1997; 402:167–72.
- 34 Smith PM, Xu H, Swoboda I, Singh MB. Identification of a Ca²⁺ binding protein as a new Bermuda grass pollen allergen Cyn d 7: IgE cross-reactivity with oilseed rape pollen allergen Bra r 1. *Int Arch Allergy Immunol* 1997; 114:265–71.
- 35 Niederberger V, Hayek B, Vrtala S et al. Calcium-dependent immunoglobulin E recognition of the apo- and calcium-bound form of a crossreactive two EF-hand timothy grass pollen allergen, Phl p 7. *FASEB J* 1999; 13:843–56.
- 36 Toriyama K, Okada T, Watanabe M et al. A cDNA clone encoding an IgE-binding protein from Brassica anther has significant sequence similarity to Ca²⁺-binding proteins. *Plant Mol Biol* 1995; 29:1157–65.
- 37 Valenta R, Duchêne M, Pettenburger K et al. Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. *Science* 1991; 253:557–60.
- 38 Valenta R, Duchêne M, Ebner C et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992; 175:377–85.
- 39 Vallier P, Dechamp C, Valenta R, Vial O, Deviller P. Purification and characterization of an allergen from celery immunologically related to an allergen present in several other plant species. Identification as a profilin. *Clin Exp Allergy* 1992; 22:774–82.
- 40 van Ree R, Voitenlo V, van Leeuwen WA, Aalberse RC. Profilin is a cross-reactive allergen in pollen and vegetable food. *Int Arch Allergy Immunol* 1992; 98:97–104.
- 41 Vallier P, Balland S, Harf R, Valenta R, Deviller P. Identification of profilin as an IgE-binding component in latex from *Hevea brasiliensis*: clinical implications. *Clin Exp Allergy* 1994; 25:332–9.
- 42 Spitzauer S, Schweiger C, Sperr WR et al. Molecular characterization of dog albumin as a crossreactive allergen. *J Allergy Clin Immunol* 1994; 93:614–27.
- 43 Breiteneder H, Pettenburger K, Bito A et al. The gene coding for the major birch pollen allergen, Bet v 1, is highly homologous to a pea disease resistance response gene. *EMBO J* 1989; 8:1935–8.
- 44 Jensen-Jarolim E, Leitner A, Hirschwehr R et al. Characterization of allergens in Apicaceae species: anise, fennel, coriander and cumin. *Clin Exp Allergy* 1997; 27:1299–306.
- 45 Swoboda I, Jilek A, Ferreira F et al. Isoforms of Bet v 1, the major birch pollen allergen, analyzed by liquid chromatography, mass spectrometry, and cDNA cloning. *J Biol Chem* 1995; 270:2607–13.
- 46 Chua KY, Kehal PK, Thomas WR, Vaughan PR, Macreadie IG. High-frequency binding of IgE to the Der p

- allergen expressed in yeast. *J Allergy Clin Immunol* 1992; 89:95–102.
- 47 Niederberger V, Pauli G, Grönlund H et al. Recombinant birch pollen allergens (rBet v 1, rBet v 2) contain most of the IgE epitopes present in birch, alder, hornbeam, hazel, and oak pollen. A quantitative IgE inhibition study using sera from different populations. *J Allergy Clin Immunol* 1998; 102:579–91.
 - 48 Niederberger V, Laffer S, Fröschl R et al. IgE antibodies to recombinant pollen allergens (Phl p 1, Phl p 2, Phl p 5, and Bet v 2) account for a high percentage of grass pollen-specific IgE. *J Allergy Clin Immunol* 1998; 101:258–64.
 - 49 Noon L. Prophylactic inoculation against hayfever. *Lancet* 1911; 1:1572–3.
 - 50 Norman PS, Lichtenstein LM, Kagey-Sobotka A, Marsh DG. Controlled evaluation of allergoid in the immunotherapy of ragweed hay fever. *J Allergy Clin Immunol* 1982; 70:248–60.
 - 51 Van Ree R, van Leeuwen WA, Dieges PH et al. Measurement of IgE antibodies against purified grass pollen allergens (Lol p 1, 2, 3 and 5) during immunotherapy. *Clin Exp Allergy* 1997; 27:68–74.
 - 52 Vrtala S, Ball T, Spitzauer S et al. 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 degranulation. *J Immunol* 1998; 160:6137–44.
 - 53 Ball T, Vrtala S, Sperr WR et al. Isolation of an immunodominant IgE-hapten from an epitope expression cDNA library; dissection of the allergic effector reaction. *J Biol Chem* 1994; 269:28323–8.
 - 54 Vrtala S, Hirtenlehner K, Vangelista L et al. Conversion of the major birch pollen allergen, Bet v 1, into two non-anaphylactic T cell epitope-containing fragments. Candidates for a novel form of specific immunotherapy. *J Clin Invest* 1997; 99:1673–81.
 - 55 Zeiler T, Taivainen A, Rytönen M et al. Recombinant allergen fragments as candidate preparations for allergen immunotherapy. *J Allergy Clin Immunol* 1997; 100:721–7.
 - 56 Ferreira F, Hirtenlehner K, Jilek A et al. Dissection of immunoglobulin E and T lymphocyte reactivity of isoforms of the major birch pollen allergen Bet v 1: potential use of hypoallergenic isoforms for immunotherapy. *J Exp Med* 1996; 183:599–609.
 - 57 Smith AM, Chapman MD. Reduction in IgE binding to allergen variants generated by site-directed mutagenesis: contribution of disulfide bonds to the antigenic structure of the major house dust mite allergen Der p 2. *Mol Immunol* 1996; 33:399–405.
 - 58 Takai T, Yokota T, Yasue M et al. Engineering of the major house dust mite allergen Der f 2 for allergen-specific immunotherapy. *Nat Biotechnol* 1997; 15:754–8.
 - 59 Ferreira F, Ebner C, Kramer B et al. Modulation of IgE reactivity of allergens by site-directed mutagenesis: potential use of hypoallergenic variants for immunotherapy. *FASEB J* 1998; 12:231–42.
 - 60 Okada T, Swoboda I, Bhalla PL, Toriyama K, Singh MB. Engineering of hypoallergenic mutants of the Brassica pollen allergen, Bra r 1, for immunotherapy. *FEBS Lett* 1998; 434:255–60.
 - 61 Valenta R, Almo S, Ball T et al. The immunoglobulin E–allergen interaction: a target for therapy of type I allergic diseases. *Int Arch Allergy Immunol* 1998; 116:167–76.
 - 62 Schramm G, Kahlert H, Suck R et al. Allergen engineering: Variants of timothy grass pollen allergen Phl p 5b with reduced IgE-binding capacity but conserved T cell reactivity. *J Immunol* 1999; 162:2406–14.
 - 63 Vrtala S, Hirtenlehner K, Susani M et al. Genetic engineering of recombinant hypoallergenic oligomers of the major birch pollen allergen, Bet v 1: Candidates for specific immunotherapy. *Int Arch Allergy Immunol* 1999; 118:218–9.
 - 64 van Hage-Hamsten M, Kronqvist M, Zetterström O et al. Skin test evaluation of genetically engineered hypoallergenic derivatives of the major birch pollen allergen, Bet v 1. Results obtained with a mix of two recombinant Bet v 1 fragments and rBet v 1 trimer in a Swedish population before the birch pollen season. Submitted.
 - 65 Pauli G, Purohit A, Oster JP et al. Clinical evaluation of genetically engineered hypoallergenic rBet v 1 derivatives by skin prick and intradermal testing: results obtained in a French population. Submitted.
 - 66 Kolbe L, Heusser C, Kolsch E. Antigen dose dependent regulation of B epsilon-memory cell expression. *Int Arch Allergy Immunol* 1991; 95:202–6.
 - 67 Sechrist H, DeKruyff RH, Umetsu DT. Interleukin 4 production by CD4⁺ T cells from allergic individuals is modulated by antigen concentration and antigen-presenting. *J Exp Med* 1995; 181:1081–9.
 - 68 Akdis CA, Blesken T, Wymann D, Akdis M, Blaser K. Differential regulation of human T cell cytokine patterns and IgE and IgG4 responses by conformational antigen variants. *Eur J Immunol* 1998; 28:914–25.