

Review

Allergy testing: the role of recombinant allergens¹⁾

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Abstract

Currently, diagnosis of type I allergy is performed using crude allergen extracts, which allow the identification of the allergen-containing source responsible for type I allergic symptoms (e.g., allergic rhinoconjunctivitis, asthma) but not the disease-eliciting molecules. With the introduction of recombinant allergens produced by molecular biology techniques, a large panel of allergenic molecules has become available. The application of these recombinant allergens for in vitro tests has led to new forms of component-resolved diagnosis (CRD) and allows the establishment of a patient's individual reactivity profile. The increasing number of recombinant allergens characterized during the last decade has allowed the development of chip-based allergy tests for simultaneous detection of up to 5000 different allergens and epitopes. The introduction of these recombinant allergen-based tests into clinical practice improves the selection of patients for traditional specific immunotherapy and allows monitoring of the immunological efficacy of specific immunotherapy by measuring allergen-specific IgG antibodies. Besides their diagnostic application, recombinant allergens and hypoallergenic derivatives thereof have also been used as vaccines in clinical trials, and recent results have shown their usefulness for the treatment of type I allergy.

Keywords: component-resolved diagnosis (CRD); cross-sensitization; hypoallergenic derivatives; microarrayed allergens; recombinant allergens; specific immunotherapy; type I allergy.

Introduction

Type I allergies represent a significant health problem. Up to 25% of the population is affected by this genetically determined hypersensitivity reaction (1), demonstrating symptoms such as allergic rhinoconjunctivitis and/or allergic asthma after contact with allergens (tree and grass pollen, mite constituents, animal hair, etc.). The clinical manifestations are triggered by the cross-linking of IgE antibodies to effector cells (mast cells, basophil granulocytes) with consequent release of inflammation mediators such as histamine, prostaglandins, and leukotrienes (2–4).

The in vitro diagnosis of type I allergy is based on the proof of allergen-specific IgE antibodies in serum, whereas provocation tests are conducted for in vivo diagnosis (5–17). The currently used allergen extracts are unsuitable for the identification of the individual allergy-eliciting molecules, because they contain non-allergenic components as well as IgE-binding allergens. On the other hand, false positive results may be ascribed to the contamination of extracts with allergens from other allergen sources (5). Only some of the proteins from an allergen source actually bind to IgE and thus result in a clinical manifestation of type I hypersensitivity.

Trials to solve the problem of diagnosis with extracts by purifying allergens from natural allergen sources have proven to be too laborious and too expensive.

Advances in molecular biology have now made it possible to recombinantly manufacture in appropriate host organisms (*E. coli*) allergy-inducing molecules that resemble the natural allergens structurally and immunologically. They can be produced with constant quality, i.e., without genetic or biological variations, and in sufficient quantities (6–20).

Determination of specific IgE antibodies against these recombinant allergens therefore allows the individual reaction profile of a patient (component-resolved diagnosis, CRD) to be classified on a molecular basis, leading to exact identification of the actual disease-inducing molecules (20).

To apply this concept to the testing of large numbers of allergens, chip systems were recently developed that permit a multi-allergen diagnosis with up to 5000 different allergens (21–24). However, recombinant allergens can also be drawn upon for the follow-up and testing of immunological efficacy by measuring specific IgG-antibody titers (25–31).

In addition, recent studies have considered the diagnostic and therapeutic application of recombinant allergens and their hypoallergenic derivatives (32, 33).

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Marker allergens and component-resolved diagnostics

Within recent years, more than 100 allergens have been identified from different biological sources (trees, grass, mites, animal hair, etc.), so that there are now numerous recombinant allergens available for routine allergy diagnosis.

To apply diagnostic information derived from the use of recombinant allergens to diagnosis of type I allergy, knowledge about certain so-called "marker allergens" is required.

The allergens already characterized can be divided into major allergens and minor allergens. Allergens from a certain allergy source to which more than 50% of sensitized patients react are designated as major allergens, and those to which less than 50% of patients react as minor allergens (34). The discrimination of cross- and co-sensitization is also useful: allergens are termed cross-reactive when they occur in different sources (i.e., trees, grass) and constitute similar three-dimensional structures and epitopes, frequently due to sequence homologies, that are recognized by the same IgE antibodies. Co-sensitization occurs when different allergens (major or minor) from various allergen sources simultaneously excite IgE reactivity in the sera of patients (35). Some cross-reactive allergens occur in biologically unrelated allergen sources and represent "marker allergens" for apparent polysensitization of a patient. Profilin, which is a protein of the cytoskeleton in all eukaryotic cells, is an example of such a marker allergen (36–38). Thus, a profilin-sensitized patient can present a reaction pattern against different allergen sources in the skin test as well as serologically, but possibly recognizing only one single highly cross-reactive molecule. By measuring specific IgE antibodies against profilins (i.e., rPhl p 12, timothy grass profilin; rBet v 2, birch profilin) cross-sensitization can be discovered as a possible cause for polysensitization. It was possible to demonstrate that profilin sensitization ranged between 5% and 40%, depending on the intensity of contact with profilin-containing allergens (39). Since most extracts are not standardized with respect to their profilin content, a patient with this reactivity profile would not benefit from conventional immunotherapy, but might exhibit an expanded allergen spectrum after treatment (40, 41).

Other allergens are produced only by pollen and not by other plant constituents (leaves, seeds). Among these are the calcium-binding allergens, with two binding sites for calcium. Due to their occurrence in pollen from trees, grass and weeds (42–44), patients that only react to the calcium-binding allergen Phl p 7 (45, 46) from timothy grass appear to be polysensitized. After identifying such, careful thought should be given to whether immunotherapy is indicated because of the lack of standardization of the extract with respect to calcium-binding allergens.

Allergens that only occur in biologically related species are termed species-specific marker allergens. These include the grass pollen allergens Phl p 1, 2, 5

and 6 that can be found solely in pollen of grass, rye, corn, and monocotyledonous plants, and therefore count as markers for genuine grass pollen sensitization (47). Since grass pollen allergens of groups 2 and 5 are expressed in most grass varieties (except Bermuda grass and corn) (48), group 6 allergens are present only in timothy grass and Kentucky blue grass (49), and group 1 and 5 allergens in all grass varieties (50), the usefulness of CRD is obvious. Therefore, genuine sensitization to grass pollen can be ascertained with the recombinant timothy grass allergens Phl p 1 and Phl p 5 (51).

Recently, marker allergens were also identified for other allergen sources and were recombinantly manufactured. The major allergens from the house-dust mite, Der p 1 and Der p 2, are antigens for serological IgE measurement that can verify house-dust mite allergy in cases of a positive skin test with mite extract and perennial allergic symptoms in the patient (52).

A highly cross-reactive allergen from this source is Der p 10, a tropomyosin that occurs in different crustaceans and can cause severe allergic reactions after oral ingestion (52).

Fel d 1, a major cat allergen, will soon be available as an additional recombinant allergen and supplemental diagnostic tool for the diagnosis of cat allergy (53).

To simplify handling of the increasing number of recombinant allergens for clinical routine, the so-called "gate keeping" concept was developed using the above-mentioned marker allergens (Figure 1) (51).

This is a diagnostic guideline for the identification of a birch pollen and/or grass pollen allergy using selected recombinant allergens that cover the IgE and T-cell epitope spectrum of the most frequent allergen sources (pollen, house-dust mite, animal hair) (51, 54).

If, for example, an IgE-mediated birch or grass pollen allergy can be verified using allergen extracts in a skin test, then the individual suitability of a specific immunotherapy can be determined with the help of recombinant allergens (Figure 1) (55).

For a birch pollen-allergic subject who reacts positively to a skin test, the allergy-eliciting molecules can now be determined in two steps. Positive testing with the major birch allergen, rBet v 1, confirms genuine sensitization against birch. In accordance with the clinical symptoms of the patient (allergic rhinoconjunctivitis in the months of March and April), specific immunotherapy with birch extract is indicated, since this contains Bet v 1 in sufficient amounts. If testing for Bet v 1-specific IgE antibodies in the patient serum is negative, then immunotherapy with birch extract is not indicated.

This approach is now also diagnostically available for an extract-based proven grass pollen allergy. When IgE antibodies against the major allergens of grass are present (recombinant allergens Phl p 1 and Phl p 5) in conjunction with historical symptoms during the grass pollen season, specific immunotherapy with grass pollen extract is indicated. However, if no

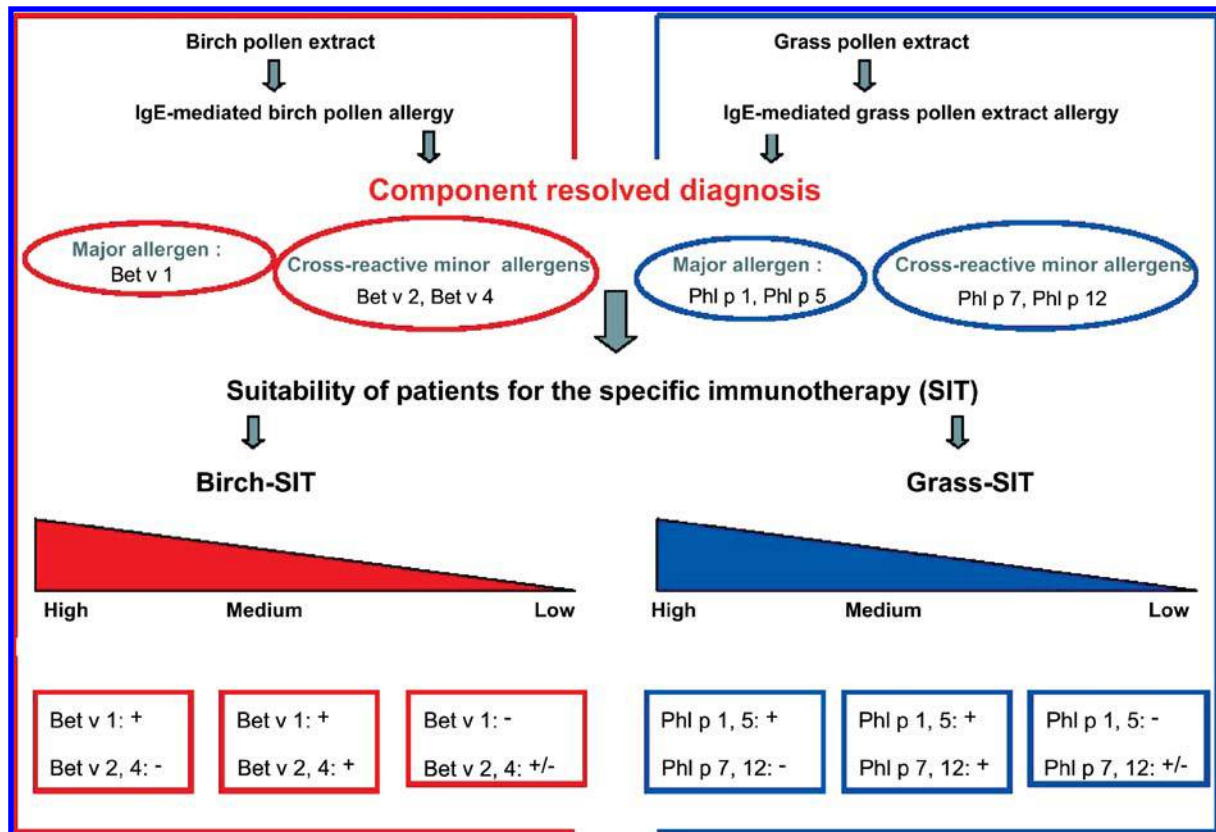


Figure 1 Guideline for the diagnosis of a birch and/or grass pollen allergy to help identify the sensitization profile of a patient on a molecular basis (allergens) and to attune therapeutic measures.

proof of a genuine grass pollen sensitization can be found, grass pollen immunotherapy is not indicated.

Soon house-dust mite marker allergens such as Der p 1 and Der p 2 will be available and will improve patient selection for specific immunotherapy (52).

Chip technology based on recombinant allergens

During recent years the number of available recombinant allergens has increased tremendously through recombinant DNS technology. A wide variety of allergens have been developed that reflect the epitope complexity of natural allergen sources. The application of these molecules in clinical practice allows the creation of an individual, patient-oriented reaction profile. Currently, allergen tests are based on single allergen determinations (ELISA, CAP-FEIA, RAST) that require a relatively high amount of serum and would be too expensive and complex for routine multi-allergen diagnosis.

The newly developed microarray technology permits application of up to 5000 allergens to detect IgE reactivity against a vast number of allergens in a single step and with a minimal amount of serum (approx. 20 µL) (Figure 2) (21–24). Using this technology, a multi-allergen test system was developed that includes recombinant allergens from tree and grass pollen, as well as from vegetable foods. To reduce signal artifacts during measurements, allergens are applied in triplicates onto the glass mount.

After incubation with patient serum, the allergen-specific IgE antibodies are verified by antibodies labeled with a fluorescent moiety. Quantification is possible with special software for data analysis.

To establish this test system, studies were conducted to compare the IgE reactivity of different recombinant allergens to systems already in use. It was possible to show that the multi-allergen chips developed are equivalent to established systems in terms of sensitivity and specificity (24).

Besides the simple handling of this test system and the much lower amount of patient serum needed, as well as the simultaneous analysis of IgE reactivity against a large number of the most diverse allergens, there is another advantage of these multi-allergen tests. While measurements of IgE reactivity by conventional methods are carried out with an obvious surplus of allergens, allergens are not offered in excess in the case of the allergen chip. This corresponds much more closely to the in vivo situation in patients, where a small amount of allergen is sufficient to elicit symptoms of a type I allergy. The application of this chip technology would not only facilitate complex analysis of serum IgE in children, but could also allow exact and inexpensive verification of existing sensitization in adults with atopic diseases (atopic dermatitis, asthma bronchiale, allergic rhinoconjunctivitis) for whom extensive allergy diagnosis would be necessary.

However, defects in carrier substances and signal artifacts currently limit the use of these chip systems

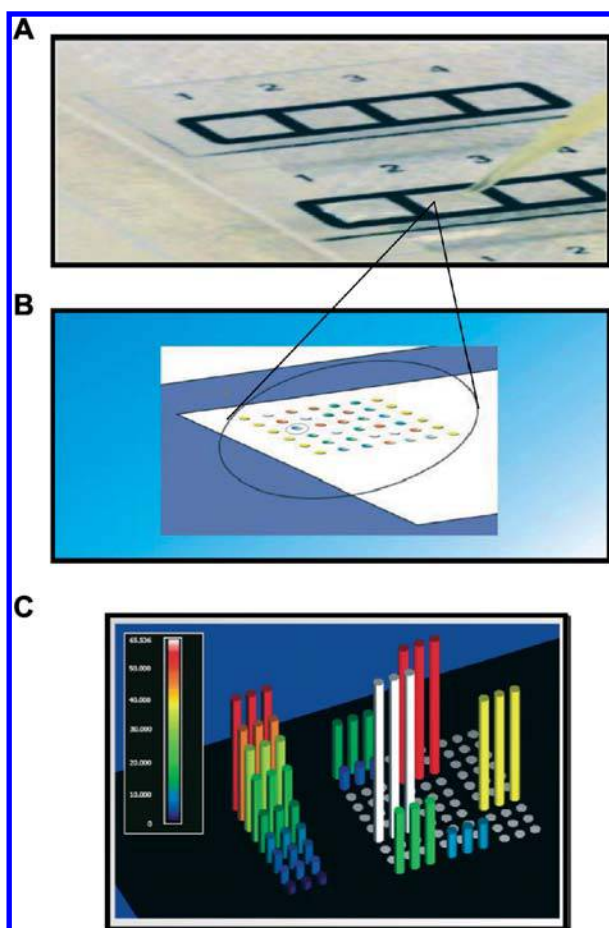


Figure 2 A multi-allergen test for the diagnosis of type I allergy. Purified or recombinant allergens are mounted onto a Teflon-coated glass carrier (A) using a fully automated spotter. On each of these marked off areas, several hundred different molecules can be spotted (B). A small amount of serum (20 μ L) is sufficient to moisturize the test area. Using a fluorescence-labeled IgE antibody, the allergen-specific IgE antibodies are detected (C). With the help of special software, the exact reaction profile of a patient can be determined and analyzed.

and further optimization processes are needed to solve these problems before this technology can be used in daily clinical routine.

Recombinant allergens for the monitoring and verification of immunological efficacy in specific immunotherapy

The goal of specific immunotherapy is to achieve "allergen-specific desensitization" by the systemic administration of increasing allergen doses. Numerous trials have proven the clinical efficacy of this approach; however, the underlying pathomechanisms remain controversial. The protective effect of "blocking IgG antibodies" on IgE-mediated effector cell activation has been demonstrated in many studies (25–33), but other mechanisms have also been discussed, such as increases in Th1-cells and a decrease in interleukin-4 (IL-4)-synthesis through T-cells (56–58), and the induction of tolerance in allergen-specific T-cells (59).

Besides the diagnostic application of recombinant allergens in guiding the choice of appropriate therapy – be it symptomatic treatment with antihistamines or specific immunotherapy – they can also be used for the monitoring of specific immunotherapy. The determination of IgE and IgG antibodies at regular intervals against single recombinant allergens instead of extracts can give clues to the success of specific immunotherapy or possibly detect recently developed sensitization (25–33, 40, 60). The determination of such antibody titers should be carried out before treatment and at regular time intervals thereafter (every 3 months), thus representing an important tool in verifying the immunological efficacy of immunotherapy.

An increase in IgG titer against the specific allergens responsible for IgE reactivity in patient serum prior to treatment would be an indication of the immunological efficacy of the therapy used (25–33). Correlation of such induced specific antibodies was recently observed in the serum and nasal secretions of patients after immunotherapy was carried out (33). In the course of studies involving serological tests based on recombinant allergens, it was also reported that some patients developed new sensitization after specific immunotherapy with total extracts (40). In a few patients with mite allergies, clinically manifest food allergies (crustaceans) were observed due to a new sensitization against tropomyosin, a highly cross-reactive mite allergen, after immunotherapy with mite extract (41).

The clinical significance of IgE sensitization is not fully understood, but is avoidable in principle.

Specific immunotherapy with recombinant allergens

Specific immunotherapy with selected extracts, besides symptomatic treatment of type I allergy, is currently the only effective causal therapy available to allergologists (61).

The inadequacy of extracts because of insufficient standardization of the allergen content has led to the development of treatment approaches with recombinant allergens that are tuned to the individual reaction profile of the patient.

Since recombinantly produced allergens correspond to naturally occurring allergens, there is also the danger of anaphylactic side effects when using these molecules in immunotherapy, as for conventional immunotherapies with total extracts. To minimize this risk, hypoallergenic variants have been manufactured with reduced allergenic activity (IgE-binding ability) (56, 62).

Several different approaches were chosen to reduce the allergenic properties of these recombinant allergens. For most respiratory allergens, the IgE reactivity is dependent on the presence of conformational epitopes on intact and structurally folded allergens (63). The immunoreaction caused by IgE antibodies in type I hyperreactivity can occur by splitting of the allergen into fragments or by the deletion of IgE-reac-

tive epitopes on the allergen. An example of this is the fragmented birch pollen allergen Bet v 1 that was first manufactured 6 years ago, which shows significantly reduced allergenic activity (64). Similar results were also observed in other studies (42, 65–69). The reduced biological activity of allergen molecules by fragmentation due to the destruction of IgE-reactive regions can be maintained by varied assembly of these fragments and can be applied in development of the so-called mosaic strategy with grass pollen allergen Phl p 2 (Mothes and Valenta, unpublished data). Other study groups showed that different allergen isotypes (differences in a few amino acids) can elicit reduced IgE reactivity (70, 71).

Surprisingly, as was observed for the birch major allergen Bet v 1, an assembly of three covalently bound Bet v 1-molecules as a trimer, with preservation of the secondary structure and the T- and B-cell epitopes, can lead to decreased activity in histamine release tests and skin tests in patients with birch pollen allergy (72). Simultaneously, it was possible to demonstrate an elevated lymphoproliferative activity in in vitro tests with T-cells of Bet v 1-allergic patients and a cytokine pattern in terms of a Th1-accentuated immunity (72).

Hybrid molecules, developed by the joining of two or three different allergens to cover the epitope repertoire of complex allergen sources, surprisingly showed increased immunogenicity in animal experiments. These data show that fusion of grass pollen allergens, in contrast to the individually applied allergens, can lead to an increase in IgG levels and T-cell response in animal tests (73).

This effect could be utilized in therapy of type I allergies to increase the immunogenicity of weaker antigens (73).

Many studies have described the advantage of recombinant allergens in vitro (IgE, IgG, lymphoproliferation, cytokine determinations, basophil release tests) and in vivo for provocation tests in patients, thereby allowing the application of these molecules in clinical diagnosis and therapy. In several studies, recombinant molecules and hypoallergenic variants with the respective wild-type allergens were compared in different provocation tests (skin, nasal and conjunctival tests) to gain information about their biological activity (14, 15, 74–76). Tests with modified derivatives of the major birch allergen Bet v 1 showed significantly reduced allergenic activity of these hypoallergenic variants (76–80). Similar results were obtained with allergens from different sources (81–83).

Recently, a clinical immunotherapy trial with over 100 patients was carried out that studied the recombinant major birch allergen Bet v 1 and its derivative allergen fragments and trimer molecule as a therapeutic measure in terms of clinical efficacy and safety (32). The immunological efficacy of these molecules was proven by a reduced seasonal IgE boost in the actively treated group, as well as by therapy-induced IgG1 and IgG4 antibodies and by the inhibition of histamine release in patients treated with protective antibodies (32, 33).

These first results demonstrate that recombinant allergens and their derivatives can be used for the establishment of allergen-specific immunotherapies.

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References

- ▶ 1. Casolaro S, Georas SN, Song Z, Ono SJ. Biology and genetics of atopic disease. *Curr Opin Immunol* 1996; 8:796–803.
2. Kay AB. Allergy and allergic diseases. Oxford: Blackwell Science, 1997.
- ▶ 3. Wills-Karp M, Santeliz J, Karp CL. The germless theory of allergic disease: revisiting the hygiene hypothesis. *Nat Rev Immunol* 2001;1:69–75.
4. Segal DM, Taurog JD, Metzger H. Dimeric immunoglobulin E serves as a unit signal for mast cell degranulation. *Proc Natl Acad Sci USA* 1977;74:2993–7.
5. van der Veen MJ, Mulder M, Witteman AM, van Ree R, Aalberse RC, Jansen HM, 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;98:1028–32.
- ▶ 6. Valenta R, Kraft D. Recombinant allergens for diagnosis and therapy of allergic diseases. *Curr Opin Immunol* 1995;7:751–6.
- ▶ 7. Valenta R, Duchene M, Vrtala S, Birkner T, Ebner C, Hirschwehr R, et al. Recombinant allergens for immunoblot diagnosis of tree-pollen allergy. *J Allergy Clin Immunol* 1991;88:889–94.
8. 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.
9. Ebner C, Szepefalusi Z, Ferreira F, Jilek A, Valenta R, Parronchi P, et al. Identification of multiple T cell epitopes on Bet v 1; the major birch pollen allergen, using specific T cell clones and overlapping peptides. *J Immunol* 1993;150:1047–54.
- ▶ 10. Valenta R, Sperr WR, Ferreira F, Valent P, Sillaber C, Tejkl M, et al. Induction of specific histamine release from basophils with purified natural and recombinant birch pollen allergens. *J Allergy Clin Immunol* 1993;91:88–97.
- ▶ 11. Sehon AH, Mohapatra SS. Induction of IgE antibodies in mice with recombinant grass pollen allergens. *J Allergy Clin Immunol* 1993;91:88–97.
- ▶ 12. Ferreira F, Mayer P, Sperr WR, Valent P, Seiberler S, Ebner C, et al. Induction of IgE antibodies with predefined specificity in rhesus monkeys with recombinant birch pollen allergens, Bet v 1 and Bet v 2. *J Allergy Clin Immunol* 1996;97:95–103.
13. Vrtala S, Mayer P, Ferreira F, Susani M, Sehon AH, Kraft, et al. Induction of IgE antibodies in mice and rhesus monkeys with recombinant birch pollen allergens: different allergenicity of Bet v 1 and Bet v 2. *J Allergy Clin Immunol* 1996;98:913–21.
- ▶ 14. Moser M, Cramer R, Brust E, Suter M, Mentz G. Diagnostic value of recombinant *Aspergillus fumigatus* allergen I/a for skin testing and serology. *J Allergy Clin Immunol* 1994;93:1–11.
- ▶ 15. Müller UR, Dudler T, Schneider T, Cramer R, Fischer H, Skrbic D, et al. Type I skin reactivity to native and recom-

- binant phospholipase A₂ from honeybee venom is similar. *J Allergy Clin Immunol* 1995;96:395–402.
16. Menz G, Dolecek C, Schönheit-Kenn U, Ferreira F, Moser M, Schneider T, et al. Serological and skin-test diagnosis of birch pollen allergy with recombinant Bet v 1, the major birch pollen allergen. *Clin Exp Allergy* 1996; 26:50–60.
 - ▶ 17. Pauli G, Oster JP, Deviller P, Heiss S, Bessot JC, Susani M, et al. Skin testing with recombinant allergens rBet v 1 and birch profilin, rBet v 2: diagnostic value for birch pollen and associated allergies. *J Allergy Clin Immunol* 1996;97:1100–9.
 18. Lynch NR, Thomas WR, Chua Y, Garcia N, Diprisco MC, Lopez R. In vivo biological activity of recombinant Der p 2 allergen of house-dust mite. *Int Arch Allergy Immunol* 1994;105:70–4.
 19. Sidoli A, Tamborini E, Giuntini I, Levi S, Volonte G, Paini C, et al. Cloning, expression and immunological characterization of recombinant *Lolium perenne* allergen Lol p II. *J Biol Chem* 1993;268:21819–25.
 - ▶ 20. Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Grönlund H. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin Exp Allergy* 1999;29:896–904.
 21. Hiller R, Laffer S, Harwanegg C, Huber M, Schmidt WM, Twardosz A, et al. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J* 2002;16:414–6.
 - ▶ 22. Harwanegg C, Laffer S, Hiller R, Mueller MW, Kraft D, Spitzauer S, et al. Microarrayed recombinant allergens for diagnosis of allergy. *Clin Exp Allergy* 2003;33:7–13.
 - ▶ 23. Suck R, Nandy A, Weber B, Stock M, Fiebig H, Cromwell O. Rapid method for arrayed investigation of IgE-reactivity profiles using natural and recombinant allergens. *Allergy* 2002;57:821–4.
 - ▶ 24. Deinhofer K, Sevcik H, Balic N, Harwanegg C, Hiller R, Rumpold H, et al. Microarrayed allergens for IgE profiling. *Methods* 2004;32:249–54.
 - ▶ 25. Ball T, Sperr WR, Valent P, Lidholm J, Spitzauer S, Ebner C, et al. Induction of antibody responses to new B cell epitopes indicates vaccination character of allergen immunotherapy. *Eur J Immunol* 1999;29:2026–36.
 - ▶ 26. Mothes N, Heinzkill M, Drachenberg KJ, Sperr WR, Krauth MT, Majlesi Y, et al. Allergen-specific immunotherapy with a monophosphoryl lipid A-adjuvanted vaccine: reduced seasonally boosted immunoglobulin E production and inhibition of basophil histamine release by therapy-induced blocking antibodies. *Clin Exp Allergy* 2003;33:1198–208.
 27. van Neerven RJ, Wikborg T, Lund G, Jacobsen B, Brinch-Nielsen A, Arnved J, et al. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4⁺ T cells by inhibiting serum-IgE-facilitated allergen presentation. *J Immunol* 1999;163:2944–52.
 - ▶ 28. Flicker S, Valenta R. Renaissance of the blocking antibody concept in type I allergy. *Int Arch Allergy Immunol* 2003;132:13–24.
 29. Nouri-Aria KT, Wachholz PA, Francis JN, Jacobson MR, Walker SM, Wilcock LK, et al. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J Immunol* 2004;172:3252–9.
 - ▶ 30. Wachholz PA, Soni NK, Till SJ, Durham SR. Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. *J Allergy Clin Immunol* 2003;112:915–22.
 - ▶ 31. Wachholz PA, Durham SR. Mechanisms of immunotherapy: IgG revisited. *Curr Opin Allergy Clin Immunol* 2004;4:313–8.
 - ▶ 32. Niederberger V, Horak F, Vrtala S, Spitzauer S, Krauth MT, Valent P, et al. Vaccination with genetically engineered allergens prevents progression of allergic disease. *Proc Natl Acad Sci USA* 2004;101:14677–82.
 - ▶ 33. Reisinger J, Horak F, Pauli G, van Hage-Hamsten M, Cromwell O, König F, et al. Allergen-specific nasal IgG antibodies induced by vaccination with genetically modified allergens are associated with reduced nasal allergen sensitivity. *J Allergy Clin Immunol* 2005;116:347–54.
 34. Marsh DG, Goodfriend L, King TP, Lowenstein H, Platts-Mills TA. Allergen nomenclature. *Bull World Health Organ* 1986;64:767–74.
 - ▶ 35. Pauli G. Evolution in the understanding of cross-reactivities of respiratory allergens: the role of recombinant allergens. *Int Arch Allergy Immunol* 2000;123:183–95.
 - ▶ 36. Valenta R, Duchene M, Ebner C, Valent P, Sillaber C, Deviller P, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;175: 377–85.
 37. Vallier P, DeChamp C, Valenta R, Vial O, Deviller P. Purification and characterization of an allergen from celery immunochemically related to an allergen present in several other plant species. Identification as a profilin. *Clin Exp Allergy* 1992;22:774–82.
 38. van Ree R, Voitenko V, van Leeuwen WA, Aalberse RC. Profilin is a cross-reactive allergen in pollen and vegetable foods. *Int Arch Allergy Immunol* 1992;98:97–104.
 - ▶ 39. Moverare R, Westritschnig K, Svensson M, Hayek B, Bende M, Pauli G, et al. Different IgE reactivity profiles in birch pollen-sensitive patients from six European populations revealed by recombinant allergens: an imprint of local sensitization. *Int Arch Allergy Immunol* 2002; 128:325–35.
 - ▶ 40. Moverare R, Elfman L, Vesterinen E, Metso T, Haahtela T. Development of new IgE specificities to allergenic components in birch pollen extract during specific immunotherapy studied with immunoblotting and the Pharmacia CAP System. *Allergy* 2002;57:423–30.
 41. van Ree R, Antonicelli L, Akkerdaas JH, Garritani MS, Aalberse RC, Bonifazi F. Possible induction of food allergy during mite immunotherapy. *Allergy* 1996;51:108–13.
 - ▶ 42. Twardosz A, Hayek B, Seiberler S, Vangelista L, Elfman L, Grönlund H, 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 Commun* 1997;239:197–204.
 - ▶ 43. Valenta R, Hayek B, Seiberler S, Bugajska-Schretter A, Niederberger V, Twardosz A, et al. Calcium-binding allergens: from plants to man. *Int Arch Allergy Immunol* 1998;117:160–6.
 - ▶ 44. Tinghino R, Twardosz A, Barletta B, Eleonora MR, Puggioni BD, Iacovacci P, et al. Molecular, structural, and immunologic relationships between different families of recombinant calcium-binding pollen allergens. *J Allergy Clin Immunol* 2002;109:314–20.
 - ▶ 45. Verdino P, Westritschnig K, Valenta R, Keller W. The cross-reactive calcium-binding pollen allergen, Phl p 7, reveals a novel dimer assembly. *EMBO J* 2002;21: 5007–16.
 - ▶ 46. Verdino P, Westritschnig K, Valenta R, Keller W. Three-dimensional structure of the panallergen Phl p 7. *Int Arch Allergy Immunol* 2003;130:10–1.
 - ▶ 47. Andersson K, Lidholm J. Characteristics and immunobiology of grass pollen allergens. *Int Arch Allergy Immunol* 2003;130:87–107.
 - ▶ 48. Niederberger V, Laffer S, Fröschl R, Kraft D, Rumpold H, Kapiotis M, 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. Vrtala S, Fischer S, Grote M, Vangelista L, Pastore A, Sperr WR, et al. Molecular, immunological, and structural characterization of Phl p 6, a major allergen and P-particle-associated protein from timothy grass (*Phleum pratense*) pollen. *J Immunol* 1999;163:5489–96.

- ▶ 50. Laffer S, Valenta R, Vrtala S, Susani M, van Ree R, Kraft D, et al. Complementary DNA cloning of the major allergen Phl p 1 from timothy grass (*Phleum pratense*): recombinant Phl p 1 inhibits IgE binding to group I allergens from eight different grass species. *J Allergy Clin Immunol* 1994;94:689–98.
- ▶ 51. Kazemi-Shirazi L, Niederberger V, Linhart B, Lidholm J, Kraft D, Valenta R. Recombinant marker allergens: diagnostic gatekeepers for the treatment of allergy. *Int Arch Allergy Immunol* 2002;127:259–68.
- ▶ 52. Pittner G, Vrtala S, Thomas WR, Weghofer M, Kundi M, Horak F, et al. Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. *Clin Exp Allergy* 2004;34:597–603.
- ▶ 53. Gronlund, H, Bergman T, Sandstrom K, Alvelius G, Reininger R, Verdino P, et al. Formation of disulfide bonds and homodimers of the major cat allergen Fel d 1 equivalent to the natural allergen by expression in *Escherichia coli*. *J Biol Chem* 2003;278:40144–51.
54. Valenta R. Diagnostic tests based on recombinant allergens: assistance for the selection of allergy therapies. In Wall K, editor: *New horizons – allergy*, Vol 1. Uppsala, Sweden: Pharmacia Diagnostics AB, 2002:1–6.
55. Westritschnig K, Kraft D, Valenta R. Rekombinante Allergene in der Diagnose der Typ I Allergie. *J Lab Med* 2002;26:120–9.
- ▶ 56. Valenta R, Ball T, Focke M, Linhart B, Mothes N, Niederberger V, et al. Immunotherapy of allergic disease. *Adv Immunol* 2004;82:105–53.
57. Jutel M, Pichler WJ, Skrbic D, Urwyler A, Dahinden C, Müller UR. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN- γ secretion in specific allergen-stimulated T-cell cultures. *J Immunol* 1995;154:4187–94.
58. Ebner C, Siemann U, Bohle B, Wilhelm M, Wiedermann U, Schenk S, et al. Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from TH2 to TH1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. *Clin Exp Allergy* 1997;27:1007–15.
59. Akdis CA, Blesken T, Akdis M, Wüthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. *J Clin Invest* 1998;102:98–106.
- ▶ 60. Van Ree R, Van Leeuwen WA, Dieges PH, Van Wijk RG, De Jong N, Brewczynski PZ, 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.
- ▶ 61. 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.
62. Valenta R. The future of antigen-specific immunotherapy of allergy. *Nat Rev Immunol* 2002;2:446–53.
- ▶ 63. Valenta R, Kraft D. Recombinant allergen molecules: tools to study effector cell activation. *Immunol Rev* 2001;179:119–27.
64. Vrtala S, Hirtenlehner K, Vangelista L, Pastore A, Eichler HG, Sperr WR, et al. Conversion of the major birch pollen allergen, Bet v 1, into two nonanaphylactic T cell epitope-containing fragments: candidates for a novel form of specific immunotherapy. *J Clin Invest* 1997;99:1673–81.
65. Ball T, Fuchs T, Sperr WR, Valent P, Vangelista L, Kraft D, et al. B cell epitopes of the major timothy grass pollen allergen, phl p 1, revealed by gene fragmentation as candidates for immunotherapy. *FASEB J* 1999;13:1277–90.
- ▶ 66. Valenta R, Vrtala S, Focke-Tejkl M, Bugalska-Schretter A, Ball T, Twardosz A, et al. Genetically engineered and synthetic allergen derivatives: candidates for vaccination against type I allergy. *Biol Chem* 1999;380:815–24.
- ▶ 67. Bhalla PL, Swoboda I, Singh MB. Reduction in allergenicity of grass pollen by genetic engineering. *Int Arch Allergy Immunol* 2001;124:51–4.
68. Vrtala S, Hirtenlehner K, Vangelista L, Pastore A, Eichler HG, Sperr WR, et al. Division of the major birch pollen allergen, Bet v 1, into two non-anaphylactic fragments. *Int Arch Allergy Immunol* 1997;113:246–8.
69. Lowenstein H, Spacholt SH, Klysner SS, Ipsen H, Larsen JN. The significance of isoallergenic variations in present and future specific immunotherapy. *Int Arch Allergy Immunol* 1995;107:285–9.
- ▶ 70. Breiteneder H, Ferreira F, Hoffmann-Sommergruber K, Ebner C, Breitenbach M, Rumpold H, et al. Four recombinant isoforms of Cor a I, the major allergen of hazel pollen, show different IgE-binding properties. *Eur J Biochem* 1993;212:355–62.
- ▶ 71. Ferreira F, Hirtenlehner K, Jilek A, Godnik-Cvar J, Breiteneder H, Grimm R, 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.
72. Vrtala S, Hirtenlehner K, Susani M, Akdis M, Kussebi F, Akdis CA, et al. Genetic engineering of a hypoallergenic trimer of the major birch pollen allergen Bet v 1. *FASEB J* 2001;15:2045–7.
73. Linhart B, Jahn-Schmid B, Verdino P, Keller W, Ebner C, Kraft D, et al. Combination vaccines for the treatment of grass pollen allergy consisting of genetically engineered hybrid molecules with increased immunogenicity. *FASEB J* 2002;16:1301–3.
- ▶ 74. Niederberger V, Stubner P, Spitzauer S, Kraft D, Valenta R, Ehrenberger K, et al. Skin test results but not serology reflect immediate type respiratory sensitivity: a study performed with recombinant allergen molecules. *J Invest Dermatol* 2001;117:848–51.
- ▶ 75. Heiss S, Mahler V, Steiner R, Spitzauer S, Schweiger C, Kraft D, et al. Component-resolved diagnosis (CRD) of type I allergy with recombinant grass and tree pollen allergens by skin testing. *J Invest Dermatol* 1999;113:830–7.
- ▶ 76. Tresch S, Holzmann D, Baumann S, Blaser K, Wuthrich B, Cramer R, et al. In vitro and in vivo allergenicity of recombinant Bet v 1 compared to the reactivity of natural birch pollen extract. *Clin Exp Allergy* 2003;33:1153–8.
- ▶ 77. Arquint O, Helbling A, Cramer R, Ferreira F, Breitenbach M, Pichler WJ. Reduced in vivo allergenicity of Bet v 1d isoform, a natural component of birch pollen. *J Allergy Clin Immunol* 1999;104:1239–43.
- ▶ 78. van Hage-Hamsten M, Kronqvist M, Zetterström O, Johansson E, Niederberger V, Vrtala S, 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. *J Allergy Clin Immunol* 1999;104:969–77.
- ▶ 79. Pauli G, Purohit A, Oster JP, de Blay F, Vrtala S, Niederberger V, et al. Comparison of genetically engineered hypoallergenic rBet v 1 derivatives by skin prick and intradermal testing: results obtained in a French population. *Clin Exp Allergy* 2000;30:1076–84.
- ▶ 80. Nopp A, Halden G, Lundhal J, Johansson E, Vrtala S, Valenta R, et al. Comparison of inflammatory responses to genetically engineered hypoallergenic derivatives of the major birch pollen allergen, Bet v 1, induces less eosinophilic activity in skin chamber fluids collected from birch pollen allergic patients than rBet v 1 wild type. *J Allergy Clin Immunol* 2000;106:101–9.
- ▶ 81. Kauppinen J, Zeiler T, Rautiainen J, Rytkonen-Nissinen M, Taivainen A, Mantylarvi R, et al. Mutant derivatives

of the main respiratory allergen of cow are less allergenic than the intact molecule. *Clin Exp Allergy* 1999;29:989–96.

- ▶ 82. Kronqvist M, Johansson E, Whitley P, Olsson S, Gafvelin G, Scheynius A, et al. A hypoallergenic derivative of the major allergen of the dust mite *Lepidoglyphus destructor*, Lep d 2.6Cys, induces less IgE reactivity and cellular response in the skin than recombinant Lep d 2. *Int Arch Allergy Immunol* 2001;126:41–9.
- ▶ 83. Bonura A, Amoroso S, Locorotondo G, Di Felice G, Tinghino R, Geraci D, et al. Hypoallergenic variants of the *Parietaria judaica* major allergen Par j 1: a member of the non-specific lipid transfer protein plant family. *Int Arch Allergy Immunol* 2001;126:32–40.