

Recombinant allergens for diagnosis and therapy of allergic diseases

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A considerable number of cDNAs coding for allergens have been isolated and expressed. Structural and immunological similarities between recombinant allergens and natural allergens indicate that a sufficient panel of recombinant allergens can be produced for diagnosis and therapy of allergic diseases. Recent studies document the successful *in vitro* and *in vivo* determination of a patient's allergen profile (allergogram) with recombinant allergens and encourage the use of recombinant allergens for specific therapy.

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Introduction

Almost a decade ago, several groups began to isolate and analyze cDNAs coding for allergens in order to determine which proteins induce IgE responses in man. Initially, astonishing sequence similarities between allergens and known proteins were found: the white face hornet venom allergen Dol m 5 [1] and the major birch pollen allergen Bet v 1 [2] showed sequence homology to pathogenesis-related plant proteins; the major house dust mite allergen Der p 1 [3] contained sequence motifs of a protease; the major *Aspergillus* allergen Asp f 1 [4] belonged to the mitogillin family; and Bet v 2 could be identified as the actin-binding protein profilin [5]. However, no significant homologs were found for the major allergens of ragweed Amb a 1 [6], cat Fel d 1 [7] and group I [8,9], group II [10,11] and group V [12–14] allergens from grass pollen. As yet, no common structural or functional features have been found among the characterized allergens, which would have allowed the prediction of whether a certain protein possesses an intrinsic property to behave as allergen.

Nevertheless, recombinant allergens proved to be useful research tools in the study of allergen-specific B-cell and T-cell responses in man. Because of structural and immunological similarities among allergens, it turned out that it might be possible to define a reasonable number of allergens for diagnosis and therapy. Indeed, it was shown that allergy diagnosis could be performed with a few recombinant tree and grass pollen allergens *in vitro* [15,16]. As a result of extensive work in the field of molecular allergen characterization, a rapidly increasing number of recombinant allergens has now become available (reviewed in [17]). This review summarizes the progress made in the field of recombinant allergens by discussing work published mostly within the review period, and gives some clues of how recombinant

allergens may be used to improve diagnosis and therapy in the future.

Isolation and characterization of recombinant allergens: cross-reactivity among allergens reduces the epitopes needed for diagnosis and therapy

The isolation of recombinant allergens requires mRNA to be prepared from the tissue in quest and reversely transcribed into cDNA. The cDNA is then used to construct a cDNA library that, depending on the system used, can be screened with antibodies or DNA probes. In the past year, a number of novel allergens were isolated by antibody screening of such expression libraries. Among the plant allergens isolated were two major allergens from timothy grass pollen (Phl p 1 [18] and Phl p 5 [14,19]), the profilin homolog from timothy grass [20], the major allergen from *Parietaria judaica* pollen (Par j 1 [21]), peanut [22], and two major allergens from Japanese cedar pollen (Cry j 1 and Cry j 2) [23,24]; the latter two allergens showed sequence similarities with fruit proteins and the major ragweed allergen family, respectively. A birch pollen allergen, Bet v 3, was identified as belonging to a novel class of calcium-binding proteins [25*]; the major olive allergen, Ol e 1 [26,27], which was obtained by PCR cloning, was found to be highly homologous to a group of pollen-specific genes; and Amb p 5 [28] isoforms were isolated directly from genomic DNA. The panel of recombinant fungal allergens [29,30*,31] and cockroach allergens [32] was increased, and a mite allergen (Der p 5 [33]) and a novel venom allergen [34] were also isolated.

The cDNA coding for the major shrimp allergen tropomyosin [35] and dog albumin [36,37] was identified

as a cross-reactive animal allergen. Several *in vitro* studies have used recombinant allergens to demonstrate cross-reactivity of IgE in allergic patients with related as well as unrelated allergens, with the outcome that extensive IgE cross-reactivities with homologous allergens in different sources were observed. On the one hand, these studies explain the clinical observation of why many individuals allergic to pollen suffer from intolerance upon ingestion of plant-derived food [38•], or why individuals allergic to dogs display allergic symptoms upon contact with different animals [36,37]. On the other hand, IgE cross-reactivities may help to select a few model allergens that bear most IgE epitopes and that can be used for diagnosis and therapy.

Recombinant allergens as tools to study specific immune responses

Before recombinant allergens can be used in the diagnosis and therapy of allergic diseases, they should be compared with natural molecules regarding their antibody-binding capacity and their ability to stimulate T cells as well as to trigger allergic effector cells (basophils). In addition, their *in vivo* activity as determined by experimental animal models, as well as by skin testing or other provocation tests in patients, has to be demonstrated. The ability to deduce the amino acid sequence from the allergen's cDNA sequence has facilitated studies of T-cell epitopes. T-cell epitope mapping studies of major grass pollen allergens [39,40] have been performed, and such T-cell peptides may be utilized for the induction of T-cell tolerance, as has been shown in mouse models [41], and discussed for human T-cell clones [42•]. Studies raising the question of whether certain T-cell epitopes may favour the onset of an allergic response, and whether others might antagonize the IgE response, did not provide evidence for such a hypothesis [43•,44•].

Whereas T-cell epitopes can be easily determined by overlapping peptides spanning the complete allergen, B-cell epitopes may belong to the continuous and discontinuous type [45]. In the case of a calcium-binding allergen, Bet v 3, evidence was provided that binding of IgE can depend critically on the allergen conformation [25•]. Continuous IgE epitopes could be determined by fragmentation of cDNAs coding for allergens [46], as well as by the construction of random fragment expression cDNA libraries [47•]. The isolation of IgE epitopes may be of therapeutical interest, because such an approach can finally lead to the identification of IgE-binding haptens that can bind IgE but that are unable to cross-link IgE bound to effector cells [47•]. Such IgE-binding haptens might then be used for a safer immunotherapy by reducing anaphylactic side effects, or to saturate allergic effector cells to prevent activation by subsequent contact with the complete allergen.

Studies on allergen-specific IgE production have been hampered so far by the difficulty of purifying sufficient amounts of defined allergens. Recent studies *in vitro* using cultured peripheral blood mononuclear cells from allergic patients and recombinant allergens have drawn attention to a pool of long-lived allergen-specific B cells in the circulation that have switched to IgE production and that appear to be unresponsive to modulators of IgE production [48,49].

Experimental animal models have proven to be extremely valuable for the *in vivo* study of allergen-specific IgE responses; the first studies with recombinant allergens in mice [50,51] and primates [52•] indicated the immunological similarity between natural and recombinant allergens. It could be demonstrated that immunization with a single recombinant allergen induced clinically relevant IgE cross-reactivity with a number of homologous allergens. Rhesus monkeys that were immunized with recombinant tree pollen allergens Bet v 1 and Bet v 2, displayed positive skin reactions to homologous allergens present in other pollen and plant-derived food [52•]. These data imply that allergic reactions to many different sources could be a result of sensitization to a few cross-reactive components.

Recombinant allergens for the diagnosis of allergic diseases

Natural allergen extracts contain a mixture of allergenic and non-allergenic components that are difficult to standardize and that, at best, allow the definition of the allergen source against which a patient is sensitized. Total extracts prepared from allergens may sometimes lack certain components either because of poor extraction conditions, or degradation during the extraction procedure. Hence, current forms of *in vitro* and *in vivo* diagnosis of allergic diseases might be improved by using recombinant allergens: recombinant grass pollen allergens have been used to identify a small panel of recombinant allergens that bind a high proportion of specific IgE [53]. The use of recombinant allergens has also allowed the determination of the patients sensitization pattern against single components. A series of studies document advances in the production of large amounts of immunologically active recombinant allergens in heterologous expression systems [54,55]. In view of these technical advances, and the increasing number of cDNAs coding for new allergens, it appears that a complete panel of recombinant allergens covering plant, mite, animal and fungal allergy will be soon available for the routine testing of patients.

Considerable progress has been made regarding the *in vivo* diagnosis of allergy using recombinant allergens. Skin testing of representative numbers of allergic patients was used to demonstrate that recombinant fungal, mite and tree pollen were equally useful to diagnose allergy compared with natural extracts [56•–59•].

Allergen-specific therapy concepts using recombinant allergens

Allergen-specific therapy of allergic diseases appears feasible in the near future for several reasons. First, an increasing number of functional recombinant allergens is now available that allows the precise determination of the patients sensitization pattern (allergen profile/allergogram) for subsequent specific immunotherapy. Second, because of extensive cross-reactivities, it may be possible to select a few recombinant model allergens that bind a high percentage of specific IgE for the usual immunotherapy regime. Finally, a number of antigen-specific therapy concepts have been developed recently, which may improve current forms of immunotherapy. The application of an allergen, a fragment, or modification thereof, will be referred to as active forms of therapy. Such concepts aim either at a prophylactic priming [60*,61,62], induction of tolerance [41], or conversion of an ongoing IgE response [63,64]. Using T-cell peptides of allergens, tolerance could be induced in mice by either injection [41], feeding [64] or inhalation [63], and *in vitro* models have been developed in order to induce non-responsiveness in human allergen-specific T-cell clones [42*]. It appears necessary, however, that cross-reactive peptides should be identified because major allergens consist of cross-reactive, as well as non-cross-reactive, T-cell epitopes, and it has been shown that isoforms of the same allergen, but that differ slightly in sequence, show different reactivity with T-cell clones [65].

In rats and mice, prophylactic application of allergen-specific $\gamma\delta$ T cells downregulated primary IgE responses [60*] indicating that specific immunoprophylaxis may prevent the onset of atopic diseases [61]. Evidence for a role of blocking antibodies that may be induced by immunotherapy comes from the observation that IgG₄ may have protective functions [66*]. Application of modified allergens or allergen-antibody complexes were reported to induce different immunoglobulin classes or to improve allergic symptoms [67,68].

In contrast to the active forms of therapy, passive strategies may aim to prevent the allergen-induced effector reaction by either applying blocking antibodies or IgE-binding haptens, which bind to IgE but are unable to cross-link IgE bound to effector cells [47*].

Conclusions

An increasing number of functional recombinant allergens has become available in recent times. Comparison of natural and recombinant allergens has revealed functional and immunological similarity. Because of extensive cross-reactivities, it appears that a few recombinant model allergens can be defined for the diagnosis and therapy of allergic diseases. In fact, a

number of recent studies has indicated that *in vitro* and *in vivo* allergy diagnosis can be performed with recombinant allergens. The major advantage of using recombinant allergens for diagnosis is that large amounts of pure and defined molecules can be produced, which allow the precise determination of the patients allergen profile (allergogram). On the basis of such reactivity profiles, those allergens representing candidates for specific therapy can be selected. Allergen-specific therapy may involve active forms of therapy that aim at prophylaxis of atopy, the induction of tolerance, or the modification of an ongoing immune response. Such attempts may be based on the application of T-cell epitopes as peptides, complete pure recombinant allergens, IgE-binding haptens, or modifications of allergens on the basis of manipulation of the allergen-encoding cDNAs (i.e. site-directed mutagenesis, which maintains T-cell reactivity but abolishes IgE binding). Passive approaches for specific therapy may focus on the prevention of the allergic effector reaction. Antibodies or genetically engineered Fab fragments might be applied locally to the mucosa to interfere with the allergen-IgE interaction by capturing allergens before they can activate effector cells. Alternatively, cocktails consisting of IgE-binding haptens might be used to saturate IgE bound to effector cells, so that contact with the complete allergens fails to trigger mediator release.

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