

Recombinant allergens for immunotherapy. Where do we stand?

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Purpose of review

This review is an update regarding the development of recombinant allergens from the laboratory bench to clinical applications. Special attention will be given to the potential improvement of allergen-specific immunotherapy through the use of recombinant allergens.

Recent findings

Currently used therapeutic allergen extracts suffer from several important disadvantages and therefore may be replaced by recombinant allergens in the near future. Recent studies indicate that recombinant allergen-based diagnostic tests can be used for selection of patients for immunotherapy and to analyse the mechanisms underlying immunotherapy.

Furthermore, recombinant and peptide technologies have been used for the generation of allergy vaccines with reduced allergenic activity. Applying the new technologies, the vaccines can be formulated to target either B cells or T cells, or both cell types. Very recently, encouraging results were obtained in an immunotherapy trial performed with genetically engineered allergens.

Summary

Recombinant allergen-based diagnostic tests will improve the selection of patients for immunotherapy. The first immunotherapy trial with recombinant allergens provides information about mechanisms underlying immunotherapy and holds promise that new types of allergy vaccines based on recombinant allergens will become available soon.

Keywords

genetically modified, hypoallergenic allergen derivatives, immunotherapy, recombinant allergen

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Introduction

Allergen-specific immunotherapy is the only causative treatment for IgE-mediated allergies. It has been shown to be effective especially in seasonal rhinitis and asthma [1]. A major limit of allergen-specific immunotherapy is the quality of the allergens used to formulate the vaccines [2]. Until now, vaccines were prepared from allergen extracts, which represent rather crude mixtures of allergens and non-allergenic materials. The first part of this review will comment on some recent papers illustrating important shortcomings of natural allergen extracts.

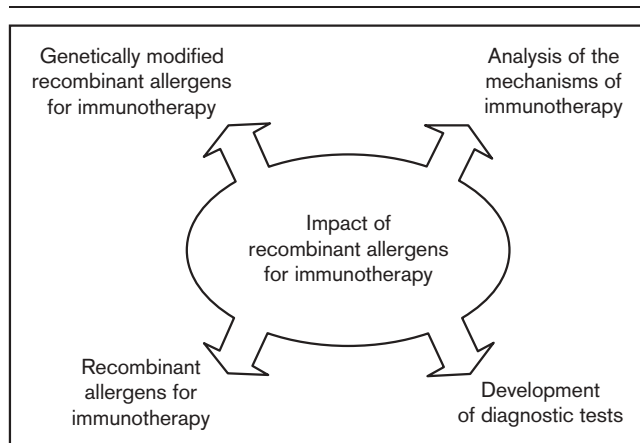
During the last 15 years, the progress of molecular biology techniques has allowed the cloning of the most common disease-eliciting allergens and the investigation of their nature [3,4]. The availability of defined allergens, in the form of recombinant molecules, has impacted on allergen-specific immunotherapy in several aspects (Fig. 1).

Component-resolved allergy diagnosis performed with allergen molecules has demonstrated that allergic patients have defined sensitization profiles [5]. The use of recombinant allergens for the analysis of allergen extract-based forms of immunotherapy has contributed to our understanding of the mechanisms underlying this treatment. We will therefore review studies which have analysed the immunological effects of allergen extract-based immunotherapy with recombinant allergens. As yet no recombinant allergen-based vaccines for specific immunotherapy are on the market but recombinant allergen-based diagnostic tests may already be able to improve the selection of patients regarding their suitability for traditional extract-based immunotherapy (Fig. 1). The usefulness of diagnostic tests based on recombinant marker allergens for the refined selection of patients for extract-based immunotherapy will be illustrated with several studies. Different approaches for immunotherapy have been developed on the basis of defined allergen molecules and their epitopes and evaluated in animal models. These studies will be summarized briefly according to the applied strategies. Finally, we will comment on the first clinical immunotherapy trial with genetically modified allergens and refer to similar ongoing work (Fig. 1).

Insights into the disadvantages of therapeutic allergen extracts gained through molecular analysis

Currently, allergy diagnosis and specific immunotherapy are performed with crude allergen extracts, which consist

Figure 1. The impact of recombinant allergens on specific immunotherapy



Recombinant allergens have impacted on several clinical applications.

of a mixture of various amounts of allergenic and non-allergenic components. The composition and allergen content of such extracts is unpredictable and depends on various factors (e.g. protein degradation, heterogeneity of allergen sources). Several recent studies [6–8] revealed that despite efforts to standardize commercial allergen extracts, there is great heterogeneity of these extracts and even the contents of major allergens may vary considerably between different batches and products. Another issue is that allergen extracts may be contaminated with unrelated allergens from other sources and hence lead to false positive test results [6]. Recently it was demonstrated that commercial allergen extracts may contain high levels of endotoxins, which adds another problem to the use of allergen extracts for immunotherapy [9•]. In this context it is noteworthy that endotoxins may influence the allergic immune response in an unpredictable manner. On the one hand it has been reported that endotoxin exposure may prevent the onset of allergic disease, whereas other studies have demonstrated that endotoxins, when used as adjuvants, may favour strong Th2 responses [10,11].

The unpredictable composition of allergen extracts may give rise to several unwanted effects during immunotherapy. A recent study [12•] investigating the IgE and IgG antibody responses in patients undergoing grass pollen-specific immunotherapy with recombinant grass pollen allergens found substantial variations of IgG responses to individual allergens, which may be attributed to under-representation or poor immunogenicity of certain components in the extracts. Other studies demonstrated that patients may develop de-novo IgE responses to allergens against which they were not sensitized before immunotherapy [13–15]. It is thus

obvious that many problems related to the use of allergen extracts for immunotherapy could be overcome by replacing the traditional extracts with defined recombinant allergens. Component-resolved immunotherapy according to a patient's sensitization profile would clearly avoid any risk of a de-novo sensitization [2]. The risk of allergenic side effects can be addressed by the use of hypoallergenic allergen derivatives produced by recombinant DNA technology or synthetic peptide chemistry [16,17•]. Moreover, it has been demonstrated that even the immunological properties of allergens can be profoundly altered by the new technologies. For example, certain derivatives were found to induce a preferential Th1 type of immune responses [18–20] and the poor immunogenicity of certain molecules could be increased by their production as hybrid molecules [21,22]. Recombinant allergen-based vaccines are not yet available on the market, but it may be possible to improve traditional allergen extract-based immunotherapy through a better selection and monitoring of patients with recombinant allergen-based diagnostic tests [23].

Recombinant allergen-based diagnostic tests to improve the indication for specific immunotherapy and for the monitoring of immunological effects induced by treatment

Allergy diagnosis with allergen extracts only allows the identification of the allergen-containing sources, whereas component-resolved diagnosis with recombinant allergens allows determination of the disease-causing molecules [2]. It has thus become possible to discriminate between two clinical conditions, namely 'co-sensitization' and 'cross-sensitization' [24]. Most patients are allergic to more than one allergen source, which may be due to sensitization to each of these allergen sources, cross-reactivity to allergens which occur in each of these sources or mixed conditions. Using recombinant marker allergens, which occur only in certain allergen sources, it is possible to diagnose genuine sensitization against an allergen source. In addition to the established clinical parameters, serological testing with recombinant marker allergens can be an important criterion for the correct indication of allergen-specific immunotherapy. Several marker allergens have been defined which can be used as diagnostic markers for sensitization to birch pollen, grass pollen, weed pollen, mite, cat and fish and hence should facilitate the correct prescription of allergen-specific immunotherapy [23,25–31]. Marker allergens may also be used to measure the immunological effects of allergen-specific immunotherapy in the form of IgG responses [12•,13,14,32,33]. It has become clear that not all types of allergen-specific IgG antibodies induced by allergen-specific immunotherapy are necessarily protective and that the protective activity may depend on many factors [34]. Nevertheless, several recent studies

point to an important role of allergen-specific IgG antibodies for the success of specific immunotherapy [12*,13,35–38]. Such IgG antibodies have been shown to block allergen-induced IgE-mediated histamine-release from basophils and to inhibit CD23-dependent IgE-mediated facilitated allergen presentation [12*,13,35,38,39]. To study the association of IgG levels with the therapeutic success of immunotherapy, component-resolved testing is of particular importance. Serum IgG antibodies may be directed against non-allergenic components from an extract or to allergens against which the patient is not sensitized, and measurements of IgG responses to crude extracts are thus of questionable value [34]. The above mentioned findings have led to a revival of the blocking antibody concept [34,37,38].

Recombinant allergens as basis for new therapeutic vaccines

The complementary DNAs coding for the most common and relevant allergens have been isolated and thus the sequences and primary structures of these allergens are available. Furthermore, the three-dimensional structures of a continuously increasing number of allergens have been and are currently being solved. Based on this knowledge it has become possible to develop a variety of molecular immunotherapy strategies. Using recombinant DNA or synthetic peptide technology it is possible to produce recombinant allergens which exactly mimic the natural wild-type allergens or derivatives which differ profoundly from the wild-type allergens in terms of physicochemical and immunological properties. A special issue of *Methods* [4*] contains a series of articles dedicated to the usefulness of recombinant allergens for clinical applications.

Wild-type-like recombinant allergens

One approach of using recombinant allergens is the application of wild-type recombinant allergens which mimic the immunological and structural properties of natural allergens. They can be used instead of allergen extracts, offering the advantage that defined molecules in defined quantities and quality can be formulated as vaccine. Wild-type-like allergens have been used in animal models to induce blocking antibodies (e.g. major fish allergen, grass pollen and *Parietaria*) [27,31,40,41] or to induce tolerance (latex and venom allergens) [42,43]. The linking of several major allergens in the form of hybrid molecules was found to increase the immunogenicity of the individual components and hence to induce higher levels of blocking antibodies in animals than vaccination with a mix of the isolated components [21,22]. At the XXIII European Congress of Allergology and Clinical Immunology, results from a recent double-blind, placebo-controlled immunotherapy trial, performed with a cocktail of recombinant grass pollen allergens, were

presented. Vaccination with the aluminium hydroxide adsorbed recombinant allergens led to a significant improvement of clinical symptoms, an increase of allergen-specific IgG₁ and IgG₄ serum levels and a blunting of the IgE response during the pollen season [44,45]. Although not yet patient-tailored, this form of treatment offers advantages with respect to pharmaceutical quality, standardization and dosage formulation of the vaccine.

Chemically modified molecules

Another approach is the linking of immunomodulatory substances to allergens to alter immune responses or to reduce the allergenic activity of allergens. Studies of the immune response to bacteria identified certain DNA motifs to be potent adjuvants which appear to activate differentiation of Th0 to Th1 cells, induce B cell proliferation, antibody production and reduce allergenicity [46,47]. One example for the use of such an adjuvant is purified ragweed Amb a 1 conjugated to CpG residues, which was shown to increase allergen-specific IgG_{2a}, indicative of a Th1 response, in animal studies, while being at the same time less allergenic than the unconjugated molecule. In a placebo controlled study by Tulic *et al.* [48], a short-course allergen-specific immunotherapy with this regimen led to changes of the nasal mucosal responses to allergen challenge (increased Th1 cytokine production and eosinophilia), and to a decrease in chest symptoms, but only during the second ragweed season. Another 2-year clinical trial with this vaccine was performed by Creticos *et al.* but is as yet unpublished. The vaccine seems to induce allergen-specific IgG responses, whereas no differences regarding the induction of the tolerogenic cytokine IL-10 in peripheral blood mononuclear cells was found between the actively-treated and placebo group [49]. Bohle *et al.* [50] recently reported the production of a recombinant fusion protein consisting of a bacterial S-layer protein from *Geobacillus stearothermophilus* linked to the major birch pollen allergen, Bet v 1. The fusion protein was shown to combine reduced allergenicity with immunomodulatory capacity towards Th1 in in-vitro studies.

Liu *et al.* [51] linked peptides of the house dust mite allergen, Der p 1, to a fungal immunomodulatory peptide. This vaccine was shown to have an anti-inflammatory effect on airway inflammation in a mouse model. Li *et al.* [52] reported that engineered peanut allergens when co-administered with *Listeria monocytogenes* protected against peanut-induced anaphylaxis in a murine model. Targeting of allergens via wheat germ agglutinin to enterocytes was attempted by Walter *et al.* and it seems that this approach can downmodulate Th2 responses [53,54]. The use of Bacillus Calmette-Guerin as a Th1-driving adjuvant to sublingual house dust mite

immunotherapy by Arikan *et al.* [55] did not improve the clinical outcome in treated patients, although an induction of IL-12 and IFN- γ production was observed [55].

Strategies targeting B cells or T cells

The idea of using a T cell epitope containing peptides to anergize or tolerize allergen-specific T cells has been taken up again and was pursued to clinical application [56]. T cell epitope-derived peptides of the major cat allergen, Fel d 1, were administered to patients and clinical as well as immunological parameters were recorded [57].

Approaches for the selective targeting of B cells are based on the identification of B cell-derived or allergen surface-derived peptides which induce blocking IgG antibodies that inhibit the binding of allergic patients' IgE antibodies to the complete allergen as well as allergen-induced effector cell activation. Such peptides have been produced according to IgE epitope mapping or structural studies of several important allergens and hence seem applicable to a broad spectrum of allergens [58–60]. Very recently a generally applicable approach for obtaining hypoallergenic IgG-inducing peptides based on allergen structures was reported [60]. The authors demonstrate that vaccination with such peptides not only blocks IgE-mediated immediate allergic inflammation but can be also used for prophylactic allergy vaccination in a murine model. Peptides inducing IgG antibodies may be also obtained by phage-display technology, which delivers short peptide-mimics of allergen-derived B cell epitopes [61,62]. The induction of blocking IgG antibodies may be achieved with such mimotopes when they are conjugated to phage particles inducing bystander T cell help [62].

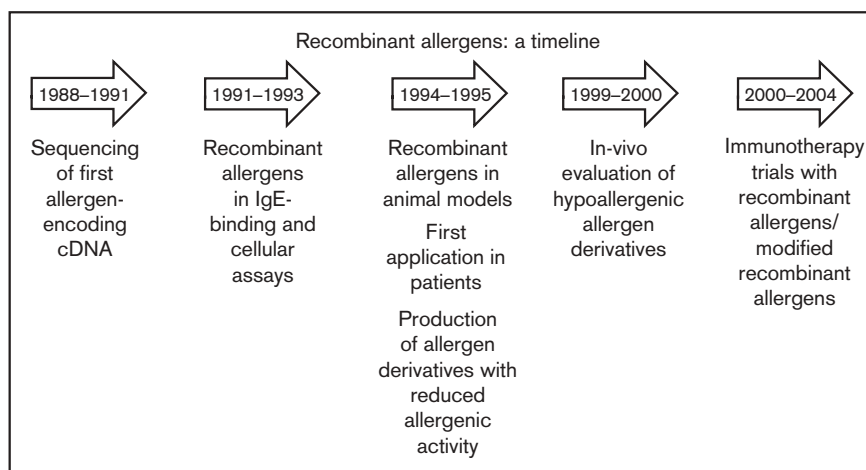
Genetically modified allergens target B cells as well as T cells

Genetically modified recombinant allergen derivatives offer several advantages [16,17•]. These molecules preserve the repertoire of allergen-specific T cell epitopes and hence can be utilized for the targeting of T cells. Furthermore, genetically engineered hypoallergenic allergen-derivatives induce blocking antibodies that inhibit the binding of allergic patients' IgE antibodies to allergens and hence also represent a B cell-based approach. Furthermore, these molecules can be engineered to reduce their allergenic activity and even to change their immunological properties. In this context, it was noted that certain genetically engineered allergens could even alter the type of immune response towards a Th1 phenotype [18–20].

The hypoallergenic derivatives of the major birch pollen allergen Bet v 1, two recombinant Bet v 1 fragments, a Bet v 1 trimer and rBet v 1 variants, have been extensively evaluated regarding safety by provocation testing in patients and their profoundly reduced allergenic activity could be confirmed [63–67]. In animals rBet v 1 fragments and trimer induced blocking IgG antibodies [18,68].

The first immunotherapy study evaluating genetically modified allergen-derivatives was in fact performed with the Bet v 1-trimer and the Bet v 1-fragments in a double-blind, placebo-controlled multicentre immunotherapy trial in 124 birch pollen allergic patients and was recently published [69•,70]. Treatment was performed with aluminium-hydroxide adsorbates of the molecules giving increasing doses (1–80 μ g) in one to two-weekly intervals as one pre-seasonal treatment course. Treatment with the hypoallergenic derivatives induced strong IgG

Figure 2. Timeline of the development of recombinant allergens from the laboratory to clinical applications



responses against the Bet v 1 wild-type allergens. Therapy-induced IgG antibodies cross-reacted with the homologous allergens from Fagales pollen and certain plant food and inhibited allergen-induced basophil degranulation. A reduction of cutaneous reactivity and improvement of symptoms was found in the actively treated patients. In addition, the rise of allergen-specific IgE production induced by seasonal allergen contact was inhibited in the vaccinated patients suggesting that this treatment also blocks the IgE memory response [69].

Conclusion

From the availability of the first allergen-encoding complementary DNAs and the first production of recombinant allergens at the end of the 1980s, recombinant allergens have made their way progressively from the bench to the clinics (Fig. 2). The usefulness of recombinant allergens for the in-vitro diagnosis of allergy was demonstrated from 1991 onwards. Skin prick tests with recombinant allergens were performed from 1994 on and from 1995 on strategies have been developed to engineer allergy vaccines based on recombinant DNA and synthetic peptide chemistry using the sequences and structures of allergens as templates. After the successful evaluation of genetically engineered hypoallergenic allergen derivatives in patients by provocation testing, the first clinical trials with the new vaccines have been initiated. Right now we see the results of the first immunotherapy trial that was conducted with genetically engineered allergens [69] and anticipate results from several ongoing trials in the near future.

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