



Recombinant Allergens: A Significant Tool of Immunotherapy

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ABSTRACT

The broad applicability of allergen-specific immunotherapy for the treatment and eventual prevention of Ig E - mediated allergy is limited by the poor quality and allergenic activity of natural allergen extracts that are used for the production of current allergy vaccines. Recombinant allergens equalling their natural counterparts have been produced for diagnosis and immunotherapy, and a large panel of genetically modified allergens with reduced allergenic activity has been characterized to improve safety of immunotherapy and explore allergen-specific prevention strategies. Recombinant allergens can be produced as defined molecules with consistent quality and unlimited amounts according to the corresponding DNA template. The recombinant allergen-based vaccination strategies will be generally applicable to most allergen sources, including respiratory, food and venom allergens and allow producing safe allergy vaccines for the treatment of the most common forms of IgE-mediated allergies.

Keywords: Allergy, Allergy vaccines, Allergen immunotherapy, Recombinant allergens.

INTRODUCTION

Traditional allergen extracts are prepared from natural allergen sources and accordingly have numerous disadvantages that cannot be overcome with existing technical means.¹ Recombinant allergens can be produced as molecules that exactly mimic the properties of the natural allergens; as modified variants with advantageous properties such as reduced allergenic activity or increased immunogenicity; or as

hybrid molecules resembling the epitopes of several different allergens to include the relevant epitopes of complex allergen sources. 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.² 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. Until now, vaccines were prepared from allergen extracts, which represent rather crude mixtures of allergens and non-allergenic materials.³ The availability of defined allergens, in the form of recombinant molecules, has impacted on allergen-specific immunotherapy in several aspects. Advantages of recombinant allergens over traditional allergen extracts are as.⁴

- They represent molecules with defined physicochemical and immunologic properties that can be modified to foster advantageous characteristics.
- The amounts can be easily controlled on the basis of mass units.
- Potencies and ratios can be exactly adjusted for each molecule.
- Represent pure molecules.
- Vaccines can be exactly tailored according to the patient's sensitization profile.
- Fit the international quality standards for vaccines.
- Can be precisely compared to give consistent and reproducible products, batches.
- Allow the precise monitoring and investigation of mechanisms underlying treatment.

There are reports that allergen extracts can be contaminated with allergens from other sources, and it has been found that patients developed new IgE reactivities under treatment with allergen extracts.⁵ Recombinant allergen-based vaccines do not contain irrelevant or unwanted components. Most recombinant allergens can be expressed in large amounts in *Escherichia coli* or insect cells at low cost without risk of containing infectious materials.⁶ 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.⁷ The first allergens to be cloned with recombinant DNA technology were Dol m 5 from the white-faced hornet (*Dolichovespula maculata*), Bet v 1 from birch pollen (*Betula verrucosa*), and Der p 1 from the house dust mite (*Dermatophagoides pteronyssinus*). Now a day, several hundred have been cloned and expressed in various systems, including bacteria, yeast, insect virus and plant. The three-dimensional structure of allergens, including Bet v 1, Der p 1, Der p 2, Bla g 2, Bos d 2, Equ c 1, and Ara h 1, has been determined by NMR techniques, x-ray crystallography, and computer-based molecular modeling.⁸ More than 400 protein or nucleotide allergen sequences are all available in different databases. Allergens constitute one of the most widely studied families of proteins in biomedical research and it is anticipated that recombinant allergens will soon become incorporated into new products for allergy diagnosis and treatment.⁹ This review draws attention mainly on studies of recombinant allergens as immunotherapeutic agents.

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 a vaccine.^{10,11} Wild-type-like allergens have been used in animal models to induce blocking antibodies (e.g. major fish allergen, grass pollen and *Parietaria*) or to induce tolerance (latex and venom allergens).¹² 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.¹³

Dose optimization for allergens:

In a few cases there is a real prospect that a single allergen or allergen derivative will suffice to achieve a substantial improvement in clinical symptoms.¹⁴ However the prevalence of sensitization and the relative contribution to the total pollen-specific IgE is taken into account.¹⁵ Allergen-specific immunotherapy is currently practiced with whole allergen extracts, and consequently, a patient who is only sensitized to the group 1 allergen is treated with a preparation containing several additional allergens in various amounts. By the same token, a preparation of recombinant allergens containing allergens to which the patient is not sensitized should not be a problem.

A further factor that might play a role in successful immunotherapy is the so-called bystander effect, which is based on the hypothesis that the immunomodulatory effect induced by treatment with one allergen has a beneficial effect on the response to others, thus providing a possible argument against the necessity to include every allergen to which a patient is sensitized.¹⁶ Firm scientific evidence is still lacking, although studies in mice suggest that antigen specificity is not a requirement for modulation of allergic responses by naturally occurring regulatory T cells. From a clinical standpoint, a bystander effect could explain the apparent ability of specific immunotherapy to prevent new sensitizations.¹⁷

Allergens as diagnostic tool

The advantages of recombinant allergens for diagnostic purposes are that they can consistently be produced in high purity.¹⁸ These reagents are more specific and more amenable to standardization in

mass units. By careful allergen selection and careful formulation of the “cocktail,” the allergenic activity of the natural product could be completely reproduced with recombinant allergens.¹⁹ The advantage of the recombinant product would be that each component would have a defined concentration and would be free of irrelevant proteins, macromolecules, or enzymes.²⁰ The purity of recombinant allergens can be established by SDS-PAGE, HPLC, and mass spectrometry. Antibody-binding activity of the recombinants can be evaluated with murine mAb in ELISA and with a large panel of human sera for IgE antibody measurements *in vitro*.^{21,22} For all the main sources of allergens, recombinant allergens can be identified that could be used in cocktails for diagnostic purposes. Exciting new developments are possible with recombinants in microchip technologies or rapid screening tests for allergy diagnosis.²³ With the use of these technologies, home-based tests for allergy diagnosis could be developed, along the lines of glucose, cholesterol, or HIV tests, to allow patients to assess their allergy status before visiting the allergist.

Critical steps in the development of recombinant allergen-based vaccines

The first important step is the selection of the predominant allergen sources. Selection criteria may include the frequency of sensitization, the clinical relevance, the magnitude of IgE responses, and the extent to which IgE epitopes are represented in a given allergen source.²⁴ By using IgE competition experiments in different populations, it has been demonstrated that the majority of IgE epitopes present in pollens of trees belonging to the order Fagales and related plant food is represented by the major birch pollen allergen, Bet v 1.10²⁵. Similar experiments have identified a panel of

recombinant timothy grass pollen allergens that contains the majority of Ig E epitopes present in grass pollens.²⁶ For the identification of cDNAs coding for the biologically most active allergen isoforms, screening protocols relying on IgE antibodies from patients with allergy may be preferred over DNA-based strategies (eg, PCR-based cloning), which may also deliver cDNAs coding for allergen isoforms with low biological activity. However, for many allergen sources (mites, molds, food), it is impossible to obtain suitable reference preparations from natural allergen sources to allow an accurate comparison of recombinant and natural allergens. Often important new allergens are discovered by molecular cloning techniques, not by protein chemical methods, because they are not present in natural allergen extracts.²⁷ In these cases, it is important to obtain as complete a panel as possible of recombinant allergens via IgE-based screening approaches and to evaluate the relevance of the individual components.²⁸ The evaluation of the individual molecules should be performed by IgE testing in large patient populations and most importantly by provocation testing because IgE serology is not always a good predictor for the allergenic activity and clinical relevance of a certain allergen molecule.²⁹ Normally, skin testing is a useful method for the evaluation of the biological activity of recombinant allergens.³⁰ Skin test studies performed in different populations may therefore be used for the identification of the clinically most relevant allergen molecules to be included in a therapeutic vaccine for a given allergen source.³¹ Lack of clinically relevant allergens will clearly reduce the efficacy of a vaccine, and inclusion of clinically not relevant molecules will make the production of the vaccine more complicated and increase costs.³² Information regarding the continuously growing number of

recombinant allergen molecules can be extracted from databases.³³ New diagnostic multi allergen tests consisting of micro arrayed marker allergens have been developed to facilitate the diagnosis and accurate prescription of immunotherapy.

Current status of recombinant allergen-based immunotherapy

The selection of the relevant recombinant allergen molecules is the prerequisite for rationale vaccine development.³⁴ Two types of recombinant allergen-based vaccines have been developed and tested in clinical trials. The first type is based on the use of recombinant allergens that equal the natural allergens (ie, recombinant wild type-based vaccines),³⁵ and the second type is based on genetically engineered/modified recombinant allergens that exhibit reduced allergenic activity.³⁶ The first immunotherapy trial with recombinant material has been performed with genetically modified hypoallergenic derivatives of the major birch pollen allergen, Bet v 1, and several analytic studies have been performed in 2001. In this double-blind, placebo-controlled trial, 124 birch pollen allergic patients were included. Active treatment was performed with aluminium hydroxide-adsorbed recombinant Bet v 1 fragments. In addition, reduced boosts of Bet v 1-specific IgE production caused by seasonal allergen exposure were noted for actively treated patients. The Bet v 1-specific IgG antibodies induced by vaccination with the recombinant Bet v 1 derivative were directed against new epitopes, demonstrating the vaccination character of the treatment.³⁷

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.³⁸ 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,³⁹ 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.⁴⁰

Therapeutic applications of recombinant allergens

Allergen immunotherapy relies on the use of high quality natural allergenic products. However, apart from improved standardization and quality control, there have been few significant innovations in allergen immunotherapy in recent years. It is possible that natural allergen products are advantageous for immunotherapy because they may contain substances that confer an adjuvant effect for down-regulating IgE responses or because they contain peptides derived from naturally digested allergens that have effects on T cells.⁴¹ The advent of recombinant allergens offers exciting new prospects for developing innovative allergen-specific treatments in which the allergens are molecular entities and any substitutions, deletions, or modifications can be precisely defined at the level of specific amino acids.⁴² There are several approaches to using recombinant allergens in immunotherapy. Cocktails of recombinant allergens could be used either together with natural allergenic products, or preferably to replace those products, in conventional immunotherapy protocols. The quality of the treatment would be improved with use of a recombinant cocktail containing uniform

allergen levels. Recombinant allergens can be engineered to produce “hypoallergens” that show reduced binding to IgE antibodies but retain T-cell epitopes. Hypoallergens have been developed for group 2 mite allergens, grass allergen Phl p 5, and peanut allergens Ara h 2 and Ara h.⁴³ Peptide-based therapy is also being pursued as an approach to treatment of peanut allergy. The ability to produce essentially unlimited amounts of recombinant allergens makes it possible to consider whether it would be possible to use prophylactic vaccination in allergic disease. With use of natural allergens, it is not possible to produce extracts that contain the milligram amounts of allergen that would be necessary for this kind of trial.⁴⁴ This approach assumes that neonatal infants are “immunologically naive,” that is, they do not make IgE responses or significant T-cell responses to allergen. There are obvious ethical issues and potential pitfalls to consider before trials of prophylactic vaccines could be initiated, as well as practical issues relating to production of recombinant allergen under good manufacturing practice conditions.⁴⁵ However, the development of recombinant allergens has generated new immunologic approaches to allergy treatment that could not previously have been considered with use of natural allergens.

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. 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.⁴⁶ Right now we see the results of the first immunotherapy trial that was conducted with genetically engineered allergens and anticipate results from several ongoing trials in the near future.

Although the recombinant allergens have been identified as a prominent tool for immunotherapy, still the researches are going on with the preparation of recombinant allergen-based vaccines for many diseases, synthesis of recombinant fusion proteins as carrying and use of these allergens in the diagnosis of allergy based syndromes⁴⁷.

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