

Editorial

Recombinant allergens: from production and characterization to diagnosis, treatment, and prevention of allergy

More than 25% of the population suffer from Type I allergy, an immunologically mediated hypersensitivity disease [1]. The symptoms of Type I allergy (e.g., allergic rhinoconjunctivitis, asthma, and atopic dermatitis) are due to the formation of IgE antibodies against per se harmless environmental antigens (i.e., allergens) [2,3]. In 1873, long before the characterization of IgE antibodies, Sir Charles H. Blackley identified allergen-containing sources (e.g., pollen) and devised methods for measuring allergen exposure and clinical challenge tests [4]. Specific immunotherapy, the only causative treatment for Type I allergy, was reported by Leonard Noon 1911, also long before the discovery of IgE antibodies ([5], reviewed in [6] and [7]). Since then, significant advances were made regarding the understanding of the immunopathological mechanisms operative in IgE-mediated allergic diseases. However, both, allergy diagnosis and immunotherapy, are still, almost as in the early days, performed with natural allergen extracts consisting of difficult to define mixtures of allergenic and non-allergenic components [8]. During the last decade several research groups have started to characterize the disease-eliciting allergens at the molecular level using molecular cloning techniques and to produce and evaluate recombinant allergens for diagnostic and therapeutic purposes (reviewed in [9]). Progress in the field of molecular allergen characterization has progressed almost on a logarithmic scale. At present, a panel of recombinant allergens has been produced which covers the epitope spectrum of the most common allergen sources.

This issue of “Methods” gives an overview about strategies for the cloning and production of recombinant allergens, their structural and immunological characterization as well as about the application of recombinant allergen-based strategies for the diagnosis, treatment, and prevention of allergies.

The paper by Slater is a state-of-the-art overview about recombinant allergens in the US. Recombinant allergens clearly offer numerous advantages over traditional allergen extracts but also will require the attention of regulatory authorities to allow doctors and patients to benefit optimally from the new technologies. A variety of strategies comprising antibody-based screening of expression cDNA libraries and PCR approaches have

been employed for the successful isolation of allergen-encoding DNA sequences in the past. The manuscript by Rhyner et al. presents an advanced technology for the efficient isolation of cDNAs coding for allergens based on phage display. With the availability of expression-, PCR- and phage display cloning the spectrum of important allergen molecules can be easily completed.

Most of the recombinant allergens have been successfully produced in large amounts as biologically active molecules in *Escherichia coli* as summarized by Wallner et al. New eukaryotic expression technologies, among them plant-based expression as described by Wagner et al. and Obermeyer et al., ensure that even difficult to express allergens (e.g., glycoproteins) can be obtained as biologically active allergens that equal their natural counterparts. The currently available protein expression technologies should thus allow us to reproduce the full spectrum of natural allergens in the form of recombinant proteins with properties equivalent to those of the natural allergens.

The three-dimensional structure of many important allergens could be revealed by X-ray crystallography and NMR analysis due to the availability of sufficient amounts of recombinant allergens. While the latter methods are time-consuming, Verdino and Keller demonstrate that circular dichroism analysis of allergens provides fast information about conformation, fold, and structural behaviour of allergens under different conditions. The fold of allergens has been found to determine its allergenic activity (reviewed in [10]). Circular dichroism therefore not only provides purely structural informations about allergens, but also allows us to make predictions about their potential allergenic activity.

First studies investigating the IgE antibody reactivity of recombinant allergens have shown that recombinant allergens resemble the IgE epitopes of natural allergens and can be used for allergy diagnosis to determine the patient's IgE reactivity profile at a molecular level [11,12]. Recent advances made in the field of chip and microarray technology have now allowed one to use microarrayed recombinant allergens for chip-based allergy diagnosis [13]. Deinhofer et al. introduce the development of chips, based on microarrayed recombinant allergens, and generate an exciting scenario for the

future developments in allergy diagnosis. Thomas et al. and Valent et al. demonstrate that recombinant allergens can also be used for the analysis of allergen-specific T cell and basophil activation and thus show that recombinant allergens promote also the analysis of cellular immune responses.

The importance of recombinant allergens for in vivo provocation testing is emphasized by Pauli and van Hage-Hamsten, who present an overview about the use of recombinant allergens for in vivo diagnosis and work done regarding the in vivo evaluation of recombinant allergen-based vaccine candidates.

The poor quality of natural allergen extracts from food has so far put strong limitations on the accurate diagnosis of food allergy. Bohle & Vieths demonstrate that recombinant food allergens will strongly improve the in vitro as well as the in vivo diagnosis of food allergy.

The use of recombinant allergens for routine diagnosis and therapy requires that accurate production, manufacture, and quality control processes be developed. Cromwell et al. report about their experience regarding the production of recombinant allergens for clinical applications. Vrtala et al. describe strategies for the production and preclinical evaluation of recombinant allergen derivatives with reduced allergenic activity for specific immunotherapy. Recombinant allergen derivatives with reduced allergenic activity have in fact been already extensively evaluated regarding their safety in clinical studies by provocation testing. A first clinical immunotherapy with genetically engineered major birch pollen allergen, Bet v 1, has been performed and several other immunotherapy trials with recombinant allergens in patients are scheduled for the end of 2003.

Based on recombinant allergens also other therapeutical strategies are currently being developed. They include the use of synthetic epitope mimicks of major allergens (i.e., mimotopes) as described by Riemer *et al.* and the development of DNA-based vaccines for allergy treatment as outlined by Hartl et al.

The issue is ended by a paper by Bhalla & Singh describing the knocking out of allergen genes from allergen sources as future scenario for the prevention of allergy. As illustrated by this issue of "Methods," recombinant allergens are on their way to fundamentally change the current practice of allergy diagnosis, treatment, and prevention. Recombinant allergen-based diagnostic tests which allow us to dissect the precise sensitization profile of allergic patients have already been introduced into clinical application. Another exciting perspective resulting from the application of recombinant allergens is the possibility to produce recombinant and synthetic allergen-derivatives with strongly reduced allergenic potential for therapeutic purposes [14]. Such hypoallergenic derivatives allow us to treat patients according to their individual sensitization profiles and may contribute to the reduction of

anaphylactic side effects in the course of allergen-specific immunotherapy. They may also represent candidate molecules for prophylactic allergy vaccination.

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