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Review

Mechanisms underlying allergy vaccination with recombinant hypoallergenic allergen derivatives

Birgit Linhart*, Rudolf Valenta

Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria

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ABSTRACT

Hundred years ago therapeutic vaccination with allergen-containing extracts has been introduced as a clinically effective, disease-modifying, allergen-specific and long-lasting form of therapy for allergy, a hypersensitivity disease affecting more than 25% of the population. Today, the structures of most of the disease-causing allergens have been elucidated and recombinant hypoallergenic allergen derivatives with reduced allergenic activity have been engineered to reduce side effects during allergen-specific immunotherapy (SIT). These recombinant hypoallergens have been characterized *in vitro*, in experimental animal models and in clinical trials in allergic patients. This review provides a summary of the molecular, immunological and preclinical evaluation criteria applied for this new generation of allergy vaccines. Furthermore, we summarize the mechanisms underlying SIT with recombinant hypoallergens which are thought to be responsible for their therapeutic effect.

1. Introduction

More than 25% of the population suffer from IgE-mediated allergy, a hypersensitivity disease affecting multiple organs [1,2]. The symptoms of allergy include mild manifestations such as allergic rhinitis, conjunctivitis but also more severe forms such as dermatitis, food allergy, asthma and life-threatening anaphylaxis [2]. Hundred years ago, in 1911, the first immunotherapy trial was published by Noon [3], who had successfully treated grass pollen allergic patients by injecting grass pollen extracts subcutaneously and found a reduction of allergic symptoms in the treated patients. In 1935 Cooke and co-workers [4] identified a protective allergen-specific factor in the serum of patients who had undergone specific immunotherapy (SIT), meanwhile known as "blocking" IgG antibodies which inhibit the binding of IgE antibodies to allergens.

Since then, specific immunotherapy has been shown to be the only disease-modifying and antigen-specific treatment of allergy, and its efficacy and long lasting effect was demonstrated in numerous clinical trials [5–7]. However, the use of crude allergen extracts as active components of allergy vaccines limits the broad application of SIT. Allergen extracts are prepared from natural allergen sources and the amount of allergen molecules in the vaccine depends on many factors, like the quality of the starting material, the extraction method and the stability of the proteins. Therefore natural allergen extracts often show great variations of allergen contents, lack important allergens and may be contaminated with allergens from other sources or other unwanted substances [8–12].

Since the late nineteen-eighties efforts were made to isolate the allergen-encoding DNAs from different allergen sources. Consequently, most of the clinically relevant allergens have been produced as pure recombinant allergen molecules which were shown to mimic the epitope spectrum of natural allergen extracts [13–15]. Allergy vaccines based on recombinant allergens were successfully tested in several clinical trials and lead to promising results in the treated patients and may soon give rise to first registered allergy vaccines which fulfil the stringent criteria required for vaccines [16–18]. However, recombinant "wildtype" allergens which possess a similar allergenic activity as the natural allergens can elicit IgE-mediated side effects which are a major problem of allergen-specific immunotherapy [19].

Early attempts to reduce systemic anaphylactic reactions in the course of treatment were made already in 1940, when adjuvanted allergen preparations came into use [20]. In 1970, David Marsh [21] proposed a method for the chemical modification of allergen extracts by aldehyde treatment, which lead to a reduced allergenic activity but maintained the immunogenicity. Due to the destruction of IgE epitopes such allergoids can be applied to patients at higher concentrations but the characterization of allergoids is extremely difficult because aldehyde treatment leads to the formation of polymers and standardization with IgE-based assays is not possible.

* Corresponding author. Tel.: +43 1 40400 5115; fax: +43 1 40400 5130.
E-mail address: birgit.linhart@meduniwien.ac.at (B. Linhart).

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Recombinant DNA technology has not only advanced the field of allergen characterization but also allowed the rational design and production of well-defined recombinant hypoallergenic allergen derivatives [13], which were even the first recombinant proteins to be tested for SIT in a clinical trial in allergic patients ten years ago [22].

2. Definition and construction of recombinant hypoallergens

Since the isolation of the first allergen-encoding cDNAs in the late nineteen-eighties a lot of information about the primary and three-dimensional structures, IgE and T cell epitopes of clinically relevant allergens has been collected [23]. Based on this knowledge approaches for the preparation of allergen derivatives with low or no IgE reactivity were tested with a view of targeting selectively the immune system [24]. Synthetic peptides containing allergen-specific T cell epitopes derived from the major cat allergen Fel d 1 were tested in first immunotherapy trials [25]. These peptides were thought to target allergen-specific T cells, as they were too small to induce IgE-mediated allergic reactions or a relevant allergen-specific IgG response. Interestingly, clinical trials using T cell peptides demonstrated then the induction of an IgE-independent allergic inflammation in the treated patients which indicated that these peptides despite their ability to induce tolerance *in vitro* can stimulate allergen-specific T cells *in vivo* [26,27]. The first genetically engineered recombinant allergen derivatives which were tested in clinical trials were constructed from the major birch pollen allergen Bet v 1 and represented two allergen-derived fragments and a recombinant Bet v 1 trimer [28,29]. The basic idea behind the construction of recombinant hypoallergenic allergen derivatives was to engineer a recombinant allergen derivative which exhibits reduced IgE-reactivity (i.e. the ability to bind allergen-specific IgE antibodies) and allergenic activity (i.e., the ability to induce IgE-mediated mast cells or basophil degranulation). The aim was to reduce IgE-mediated side-effects in the course of immunotherapy. Despite the fact that most of the hypoallergens lost their native conformation, it has been found that they induce upon immunization allergen-specific IgG antibody responses, which interfere with the IgE recognition of wildtype allergens [30]. Most of the recombinant hypoallergens are made in a form to preserve most allergen-specific T cell epitopes and they generally seem to induce less allergen-specific IgE antibodies than the corresponding wild type allergens upon immunization (i.e., reduced allergenicity which refers to the ability to induce an allergen-specific IgE antibody response upon allergen contact) [30,31]. Table 1 provides a summary of the types of recombinant allergen derivatives which have been made.

The reduction of IgE reactivity can be obtained either by mutation of the amino acid residues involved in IgE-binding, or by the disruption of the three-dimensional structure of the allergen [23]. The latter approach is based on the finding that the IgE antibody response to respiratory allergens is mainly directed to conformational epitopes, probably because during allergic sensitization the immune system recognizes these allergens as intact proteins when they are taken up via the respiratory mucosa [32]. Several studies have demonstrated that the intact conformation of allergens is crucial for IgE binding and that the disruption of the three-dimensional fold leads to a reduction or loss of the IgE binding capacity [32]. The production of allergen-derived fragments lacking IgE-reactivity as described for the major birch pollen allergen Bet v 1 [28] can in principle by applied to other allergens [33–35], but may be limited by the lower immunogenicity of these fragments, resulting in a low allergen-specific IgG response. Therefore this strategy was further developed towards the construction of mosaic molecules, consisting of re-assembled allergen-derived fragments within one molecule [31,36–39]. These mosaics retained the lack of IgE-binding activity of their components due to the loss of their three-dimensional structure but were able to induce robust allergen-specific IgG antibody responses. The fusion of a hypoallergenic mosaic molecule and an allergen fragment, derived from different major grass pollen allergens, respectively, was shown to increase the immunogenicity of the included molecules [40]. The concept of producing hybrid molecules consisting of hypoallergenic allergen-derived fragments is especially attractive for the construction of hypoallergenic vaccines for complex allergen sources, like grass pollen and house dust mite, which contain several major allergens because it allows to increase the immunogenicity of the vaccine and at the same time to reduce the number of molecules which need to be included in the vaccine ([40–42], reviewed in [43]).

Interestingly it was found, that oligomerization of allergens may yield hypoallergenic oligomers for certain allergens by a mechanism of altered IgE epitope presentation [44]. The fusion of three full-length copies of an allergen-encoding gene was shown for the major birch pollen allergen Bet v 1 and resulted in a Bet v 1 trimer [29]. The trimer turned out to be a highly immunogenic protein in allergic patients but showed a strongly reduced allergenic activity [22,45,46]. When the hypoallergenic nature of the molecule was further analyzed it was found to form high molecular weight aggregates leading to altered presentation of IgE epitopes to effector cell-bound IgE which was proposed as a mechanism responsible for the hypoallergenicity [44].

Several hypoallergens were obtained by insertion of mutations in the wildtype sequence [47–63]. Hypoallergens derived from the major grass pollen allergen Phl p 5 and Phl p 6 were generated by introducing deletions in order to disrupt the protein structure [60,63]. Amino acid exchanges represent a widely used method for the generation of hypoallergens, whereby different strategies have been applied. The mutation of cysteins which are involved in the formation of disulfide bonds stabilizing the protein structure has been shown to disrupt the allergen conformation and IgE epitopes and abolish IgE recognition. Allergens belonging to the family of Ca-binding proteins nicely illustrate the relation between IgE-binding and allergen structure. As their conformation depends on the presence of protein-bound calcium, it was shown that depletion of calcium from these allergens lead to the loss of IgE reactivity [64]. The same effect was observed when mutating the calcium-binding domains, as shown for the cross-reactive grass pollen allergen Phl p 7 and the major fish allergen Cyp c 1 [55,58].

The strategies described above allow the production of well-defined molecules (regarding their biochemical and immunological characteristics as well as the production process) in a stable and reproducible way, which cannot be achieved by chemical modifications of the corresponding recombinant wildtype allergen [65,66].

Beside the loss of conformation and therefore IgE reactivity, amino acids, which are directly involved in the allergen–IgE interaction, were targeted by site-directed mutagenesis [67,68]. However, IgE epitopes are difficult to identify, as IgE directed to a conformational epitope normally does not bind to linear peptides [69]. Therefore more elaborated methods for IgE epitope mapping studies have to be applied, like the co-crystallization of allergen-IgE complexes or competition experiments using antibody probes

Table 1
Types of recombinant hypoallergenic allergen derivatives.

	References
Fragments	[28,33–35]
Oligomers	[29]
Mosaic molecules/hybrids	[31,36–42,59]
Mutants	[47–63]
Denatured proteins	[65–68]