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Recombinant allergen-based provocation testing

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ABSTRACT

Over the last 25 years, recombinant allergens from all important allergen sources have been cloned and are now available as recombinant proteins. These molecules can be produced in practically unlimited amounts without biological or batch-to-batch variability. It has been shown in provocation tests that recombinant allergens have similar clinical effects as their natural counterparts. With the help of these tools it is possible to reveal the precise reactivity profiles of patients and to uncover and differentiate cross-reactivity from genuine sensitization to an allergen source. Although it has been shown some time ago that it would be possible to replace crude allergen extracts with recombinant allergens for skin prick testing, and even though the use of allergen components can improve routine diagnosis, these tools are still not available for clinical routine applications. The use of provocation tests is a crucial step in the development of new, hypoallergenic vaccines for therapy of allergic disease. Here we describe important provocation methods (skin prick test, intradermal test, atopy patch test, nasal provocation, colonoscopic provocation test) and give an overview of the clinical provocation studies which have been performed with recombinant allergens so far.

1. Introduction

Over the past 25 years, recombinant forms of most important allergens have been produced and have been shown to be equal to their natural counterparts regarding their ability to bind IgE antibodies, stimulate T cells and induce allergic reactions [1,2]. Recombinant allergens are defined molecules which can be produced in a highly purified and controlled manner, thus avoiding batch-to-batch variability. This is opposed to natural allergen extracts, which contain most often several different allergens in varying concentrations [3–5] as well as a high number of non-allergenic components. The composition of allergen extracts depends on several not manipulable factors during the production of the natural source material [6,7]. Furthermore, standardization of allergen extracts can only be made for one major allergen, while the composition of other components remains unchanged [8]. Recombinant allergens have been shown to be able to complement or replace natural allergen extracts for diagnosis [9], and highly sophisticated *in vitro* diagnostic test systems have been developed which allow the precise analysis of the reactivity profiles of allergic patients [10–12]. At present, a combination of tests based on natural allergen extracts and component resolved testing is used for diagnosis of allergy in routine settings.

Specific immunotherapy is the only allergy treatment which is able to change the course of allergic disease [13,14]. Based on the knowledge of the precise immunological and structural properties of allergens and the location of IgE epitopes, recombinant allergen derivatives which have a reduced ability to induce effector cell degranulation have been produced, with the goal to improve treatment success and reduce side effects of immunotherapy [15,16]. The clinical characteristics of promising vaccine candidates need to be evaluated not only in *in vitro* test systems but also directly in allergic patients, using provocation testing [17–19]. According to a guideline by the European Medicines Agency (EMA) published on June 1st in 2009 (http://www.ema.europa.eu/pdfs/human/ewp/1850406enfin.pdf), new allergy vaccines do not need to undergo a classical phase I clinical study in healthy non-allergic subjects. This clinical phase is usually replaced by a provocation study in allergic subjects, e.g., a skin test study, which is immediately followed by a phase II study in allergic patients.

The number of published studies employing provocation testing with recombinant allergens has declined substantially over the past few years. This can be explained by the implementation Commission Directive 2003/94/EC (Medicinal Products for Human and Veterinary Use. Eudralex), which regulates the Good

Abbreviations: GMP, good manufacturing practice; SPT, skin prick test; APT, atopy patch test; NPT, nasal provocation test; IDT, intradermal test; COLAP, colonoscopic allergen provocation test; AD, atopic dermatitis; GI, gastrointestinal.

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Manufacturing Practice (GMP) in the EU, thus prohibiting the approval of clinical studies with non-GMP produced recombinant allergens. As GMP production of allergens is both elaborate and costly, only few recombinant allergens meeting these criteria are currently available and only few provocation studies with recombinant allergens were initiated after that time point. However, it is to be expected that the number of recombinant allergens produced in GMP quality will increase over the next few years and thus the number of provocation studies with recombinant allergens will rise again. In particular, there remains the necessity of clinical evaluation of new recombinant allergen vaccines before they can be put into use for subcutaneous or sublingual treatment of allergic patients. This review will summarize provocation methods which can be and have been performed with recombinant allergens, mainly focussing on skin prick and intradermal testing, atopy patch testing, nasal provocation testing and colonic provocation testing. Bronchial and conjunctival provocation testing will both be only shortly addressed.

2. What can be gained by using allergen components for provocation testing?

With the wide availability of allergen components for measurement of allergen-specific IgE, the routine diagnostic spectrum in *in vitro* tests has changed. Component-resolved testing allows the identification of patients who are genuinely sensitized to an allergen source and those who have positive skin reactions merely because they are sensitized to a highly cross-reactive panallergen [20]. Although many skin test studies and other provocation studies have already been performed with recombinant allergens (Tables 1–3) and although the advantages of skin testing with recombinant allergens have been recognized many years ago [21], and even though standardization of allergen extracts has remained a difficult problem [8], 25 years after the first allergen was produced in a recombinant form even the most relevant allergen components are still not commercially available for biological testing. This can be attributed to the fact that test substances based on recombinant allergens legally need to undergo far more rigorous, elaborate and costly studies than those based on natural allergen extracts [22].

Another important application of recombinant allergens or allergen components is to study the clinical relevance of allergen components. It has been shown previously that the IgE binding capacity of an allergen alone does not predict its ability to induce allergic responses [23]. This is of particular importance for the design of new allergy vaccines. In this context, it has been shown that group 4 and group 13 grass pollen allergens have ninefold smaller allergenic activity than other grass pollen allergens (group 1, 2 and 5 allergens) and are therefore not essential components of therapeutic vaccine formulations against grass pollen allergy [24]. Furthermore, provocation tests have proven to be valuable for the evaluation of new therapeutic vaccines which have altered IgE binding capacity and allergenic activity (Table 2).

A comparison of possible advantages and problems associated with the use of natural allergen extracts and recombinant allergens for biological testing are listed in Table 4.

3. Skin prick and intradermal testing

Skin prick tests (SPT) and intradermal or intradermal skin tests (ICT) were introduced by Blackley in 1865 [25] and have since then served as an important tool in diagnosis immediate-type allergic reactions. They are easy to perform, inexpensive, safe and allow a visualization of sensitization within 15–20 min. They are performed by introducing small amounts of allergen into the dermis [26]. In the skin of allergic subjects, effector cells are armed with allergen-specific IgE that is bound to their high affinity receptor, FcεRI. Upon contact with allergen, cross-linking of IgE occurs and leads to release of mediators (histamine, tryptase, TNF-α, prostaglandins, leukotriens, IL-4, and others [27,28]). The released mediators cause vasodilatation and increase vascular permeability of the skin, thus resulting in tissue edema and the development of the typical "wheal reaction" as well as localized erythema caused by vasodilatation. In skin prick tests, mainly the size of the wheal determines whether a skin prick test reaction is regarded as positive or negative, while the erythema is usually not accounted for [29,30]. A late phase reaction may occur one to two hours later, peaking at 6 to 12 h and usually diminishes within 48 h [31,32].

It needs to be borne in mind that results from skin prick testing and the measurement of allergen-specific IgE in the serum do not always correlate and that subjects with positive skin reactions do not necessarily suffer from allergic symptoms [33–35].

A number of recent skin test studies have explored the usefulness of three allergen components for the diagnosis of food allergy [40–43]. The use of recombinant allergen components would be particularly useful in food allergy as the detection of potential pollen-food cross-reactivity is important and food allergen extracts are often unstable and unreliable. In a study by Viera et al. [42], natural profilin (Phl p d 2) from date palm extract, the major apple allergen, Mal d 1, from apple extract and a peach LTP commercial extract which was shown to lack other allergens were used for skin testing and compared with IgE reactivity to recombinant Bet v 1, Bet v 2, Phl p 12 and Pru p 3. The authors found that sensitization to pan-allergens in children with fruit and vegetable allergy was common and that using allergen components would be a simple and feasible way of improving allergy diagnosis. In another study, Asero et al. studied the clinical relevance of positive skin prick tests to the same three allergen components in pollen allergic patients [43]. The authors confirmed that the clinical relevance of hypersensitivity to pan-allergens is often limited in patients with respiratory allergy.

3.1. Methods of skin testing

3.1.1. Skin prick tests

Skin prick testing is a routine method which has recently been extensively reviewed [30,44] and will therefore not be described in detail in this review, which will focus on the particularities of skin prick testing with recombinant allergens. In short, a skin prick test is performed by applying the allergen solution on the volar forearm or, if this is not possible, the back of the patient. A lancet is passed through the drop and inserted into the skin. The wheal and flare reaction is interpreted after approximately 15 min.

3.1.2. Intradermal tests

Intradermal skin tests have been used for the biological evaluation of recombinant allergens and for validation of genetically engineered hypoallergenic derivatives (Tables 1 and 2). Before the test, patients are advised to stop the use of certain medications (Table 5). The recombinant allergen solution is injected intracutaneously from a 0.5 or 1.0 ml plastic syringe through a 26-gauge needle. Between 0.02 and 0.05 ml of the allergen solution is injected into the skin to produce an intradermal bleb approximately 3 mm in diameter. Wheal reactions less than 5 mm are regarded as negative [45,46]. In experienced hands, the intradermal skin test is more reproducible than SPT, but a higher level of technical skill is required [47,48]. The advantages of the intradermal test are a higher sensitivity, disadvantages are that the test is painful, more laborious to perform and more often produces false-positive reactions. Furthermore, it has an increased risk of systemic allergic reactions as compared to skin prick testing [45].