

Allergy Diagnosis, Allergen Repertoires, and Their Implications for Allergen-Specific Immunotherapy

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Allergic and asthmatic diseases represent a big health problem in industrialized countries where as many as 20% to 30% of the population sustain type I allergic symptoms such as rhinitis, conjunctivitis, atopic dermatitis, or bronchial asthma [1,2]. These diseases represent an important cause of morbidity and, occasionally, mortality [3]. Atopic disorders have become a relevant socioeconomic problem causing a health care burden to society in the order of billions of US dollars [4,5]. A common hallmark of these diseases is the production of allergen-specific IgE against normally innocuous environmental antigens. In sensitized individuals, allergen re-exposure induces cross-linking of high-affinity FcεR1 receptor-bound IgE on effector cells and immediate release of anaphylactogenic mediators [6]. The clinical symptoms deriving from mild type I hypersensitivity reactions are not life threatening and manifest mainly as generalized urticaria, itching, constriction in the chest, nausea, vomiting, angioedema, and abdominal pain. Severe forms manifest as dyspnea, wheezing, and respiratory and cardiovascular symptoms up to shock with loss of consciousness and are associated with a risk of fatal reaction [7].

Current drugs for allergic diseases, such as antihistamines, corticosteroids, bronchodilators (β-adrenergic receptor antagonists), and anti-IgE antibody ther-

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apy, treat allergy symptoms and concomitant inflammatory reactions [8] and require a long-term recurrent administration of the drugs [9,10]. Allergen-specific immunotherapy (SIT) is the only treatment able to cure allergic diseases and has been shown to be successful in insect venom allergy [11] and patients sustaining allergic rhinitis [12] but not in other complex allergy syndromes such as eczema, food, or mold allergy [13]. There is a need for defining new therapeutic targets and strategies to allow a more efficient treatment of allergic diseases justified by the severely impaired quality of life of affected individuals and the annual costs related to the treatment of allergy and asthma [3–5]. Hyposensitization (immunotherapy) is the practice of administering gradually increasing quantities of an allergen extract to an allergic individual to ameliorate the symptoms associated with subsequent exposure to the causative allergen. Standardized allergen extracts have been designated “vaccines” in a recent World Health Organization position article on allergen immunotherapy [14]. Currently, allergen-SIT is performed using extracts from natural sources containing insufficiently characterized allergen mixtures and unwanted nonallergenic components. Immunotherapeutic treatment of allergic disease spans a period of 3 to 5 years. Unfortunately, allergen extract-based SIT can induce anaphylaxis as well as new IgE responses to allergens contained in the extract [15,16]. The development of new strategies for disease management requires a detailed understanding of the molecules able to elicit allergic reactions and the immunologic mechanisms underlying the pathology of type I hypersensitivity reactions.

Physiologic background of allergic diseases

The breakthrough in understanding hypersensitivity reactions was the discovery of the “reaginic antibody,” termed *immunoglobulin E* (IgE), by Ishizaka and Ishizaka [17] more than 30 years ago. Allergy is caused by the overproduction of allergen-specific IgE in response to the exposure to allergens [1]. Pollens, foods, dust mites, animal dander, fungal spores, and insect venoms represent the most important sources of sensitization leading, after re-exposure, to a variety of different clinical manifestations classified on the basis of their immunologic pathogenesis. The classification of hypersensitivity reactions into types I to IV [18] is widely accepted and used in clinical practice. In this context, the term *allergy* is used in a restricted sense to describe type I hypersensitivity reactions referring to damage caused by IgE antibody-mediated immune responses. IgE, a class of immunoglobulin normally present in the serum at low concentrations, is produced by IgE-secreting plasma cells, which express the antibody on their surface at a certain stage of their maturation [19]. For reasons still not well understood, allergic individuals produce increased amounts of IgE with binding specificity for ordinarily innocuous antigens to which they are sensitive [20,21]. The allergen-specific IgE circulates in the blood and binds to IgE-specific receptors on the surface of basophils in the circulation and mast cells along mucosal linings and

underneath the skin. During re-exposure, the inhaled or ingested allergens bind to IgE on mast cells and basophils, cross-link the IgE molecules, and aggregate the underlying receptors, triggering the effector cells to release histamine and other pharmacologic mediators causing symptomatic responses [22]. Systemic reactions to allergens may involve the cutaneous, gastrointestinal, respiratory, and cardiovascular systems, presenting in some cases as anaphylaxis [23].

Diagnosis of allergy and allergen repertoires

The diagnosis of an allergy is based on clinical history, skin test reactivity to the offending allergen, and in vitro determination of allergen-specific IgE antibodies in serum [24]. The results of in vivo and in vitro diagnostic procedures strongly depend on the allergen preparation used [25]. Despite many commercially available allergen extracts, only a few standardized extracts recognized by the US Food and Drug Administration are currently available [26]. The accuracy of skin tests in vivo and IgE determinations in vitro can be compromised if conducted with different protocols or with allergen extracts having insufficient quality control. Given the respective trajectories for technologic advancement, quantification, and quality control, recombinant allergens producible as single highly pure reagents offer a standardized approach for the diagnosis and treatment of allergic diseases [25]. Theoretically, single recombinant allergens can also be used as vaccines for an allergen-SIT; however, the success of such a treatment will depend on a patient-specific component-resolved diagnosis of the molecular structures to which a given individual is sensitized to avoid new sensitizations to additional allergens present in the extracts used for therapy [15].

The major allergens from the most prevalent allergenic sources (eg, pollens, mites, animal dander, food, molds, and insect venoms) have been identified and produced by recombinant DNA technology, allowing the assembly of panels of recombinant allergens reconstructing the epitope complexity of the corresponding allergen extracts [27–31]. The number of different allergenic structures present in different sources is highly variable. Some extracts such as grass and tree pollens or bee venoms contain only a few allergens, and it has been shown in both cases that a component-resolved allergy diagnosis based on recombinant allergens, approaching the sensitivity of the diagnoses obtained with commercially available allergen extracts, is feasible [32,33]. Other allergenic sources such as mites or molds contain complex allergen repertoires spanning several dozen different molecular structures [28,34,35]. *Aspergillus fumigatus* with an estimated allergen repertoire of more than 80 different structural entities represents the most complex system described thus far [36]. Nevertheless, sensitization to *A. fumigatus* using skin tests and serology can be diagnosed with a high sensitivity and specificity using recombinant allergens [37,38].

In general, it is widely recognized that the diagnosis of allergic diseases based on recombinant allergens is superior to extract-based diagnosis in terms of

specificity [39]. The technologic achievements in producing recombinant proteins will allow one to reconstruct the epitope complexity of natural extracts by mixing up panels of single recombinant allergens. The big challenge in view of diagnostic and therapeutic applications of recombinant allergens will be to estimate the size of the epitope repertoire needed to substitute allergen extracts. Bioinformatics analyses based on structural motifs [40] and BLAST similarity search methods [41] involving 101,602 and 135,850 protein entries deposited in the Swissprot database predict 4093 (4%) and 4768 (3.5%) different potential allergen structures, respectively; therefore, one can assume that the size of the allergen repertoire involved in eliciting allergic symptoms is in the range of 5000 different structures. Not all structures will be required for diagnostic and therapeutic applications, because many allergens can be grouped in pan-allergen families sharing cross-reactive epitopes [42–45]. Cross-reactivity will reduce drastically the number of epitopes needed for a careful diagnosis of allergic conditions. The rapidly increasing knowledge of primary and three-dimensional structures of allergens [46] will speed up theoretical and practical progress in defining the number of structures/epitopes needed for a component-resolved diagnosis of allergic diseases, forming the basis for the development of rational therapy approaches. Moreover, rapid progress in developing microchip-based diagnostic procedures [47,48] will allow parallel multiplexed analyses of serum samples at low cost, a prerequisite to establish the use of recombinant allergens in clinical routine assessments.

Current forms of allergy treatment

Current drugs for allergic diseases, such as antihistamines, corticosteroids, and bronchodilators (β -adrenergic receptor antagonists) treat allergic symptoms and concomitant inflammatory reactions [8]. Although pharmacotherapeutic ap-

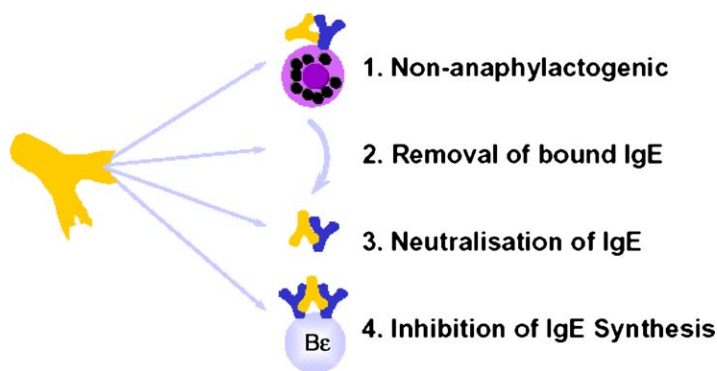


Fig. 1. Properties of antihuman IgE monoclonal antibodies used in passive anti-IgE therapy. (*Adapted from Marti P. New perspectives for allergy diagnosis and therapy [PhD thesis]. University of Berne, 2006; with permission.*)

proaches are effective in controlling symptoms, these therapies have a limited capacity to alter the natural course of allergic diseases, and discontinuation of medication results in the redevelopment of symptoms on re-exposure to the offending allergen. A new approach in treating allergic diseases and asthma consists of the administration of anti-IgE antibodies to out-compete IgE binding to the Fc ϵ RI and Fc ϵ RII receptors (Fig. 1), preventing cross-linking and release of mediators [9]. This type of treatment requires a long-term recurrent administration of the drug [10]. SIT, the only treatment to cure allergic diseases, is performed using allergen extracts [14]. Unfortunately, SIT can induce new IgE responses to allergens contained in the extract [15] as well as anaphylaxis [49]. A long-term therapy consisting of the practice of administering subcutaneously gradually increasing doses of an allergen extract to an allergic individual, SIT is most efficiently used in allergies to insect venoms [11] and allergic rhinitis [12,50]. It is not efficient in other complex allergic syndromes, such as eczema, food, or mold allergy [13].

New trends in allergen-specific immunotherapy

Recent advances in understanding the immunologic mechanisms underlying allergic diseases and the molecular characterization of allergen repertoires have greatly expanded the potential therapeutic options for future use. Nevertheless, only a few new drugs have reached clinical application, and none provides long-term immunomodulatory effects [51]. Immunotherapy through its capacity to produce a long-lasting, antigen-specific, protective immune response is the only etiologic treatment that offers the possibility of curing or even preventing atopic diseases [52,53]; however, the potential for severe side effects associated with conventional immunotherapy using whole allergen extracts limits its widespread use, especially in highly sensitized individuals [7,54]. The major problem has been recognized to be related to IgE-facilitated allergen presentation mediated by allergen-specific B cells [55]. B cells can present low concentrations of allergen to T cells (Fig. 2), inducing high interleukin-4 (IL-4) but little or no interferon- γ (INF- γ), favoring Th2-biased immune responses. By contrast, the use of monocytes and macrophages as antigen-presenting cells and high doses of allergen results in high INF- γ but low IL-4 synthesis, favoring an immune deviation toward a balanced Th0/Th1 immune response associated with blocking antibodies [55,56]. As a consequence, the high doses required for successful immunotherapy might not be reached using native allergen extracts [55]. Novel strategies to minimize the side effects and improve the efficacy of immunotherapy are of considerable interest in the treatment of atopic diseases. Promising animal and human studies using approaches such as peptide immunotherapy, DNA vaccination, CpG oligonucleotides, and modified allergens suggest that it might be possible to prevent or cure atopic disorders in an efficient way [52,53]. Nevertheless, the actual vaccination concepts still suffer from an extremely long treatment period as true for classical SIT, and the development of improved and

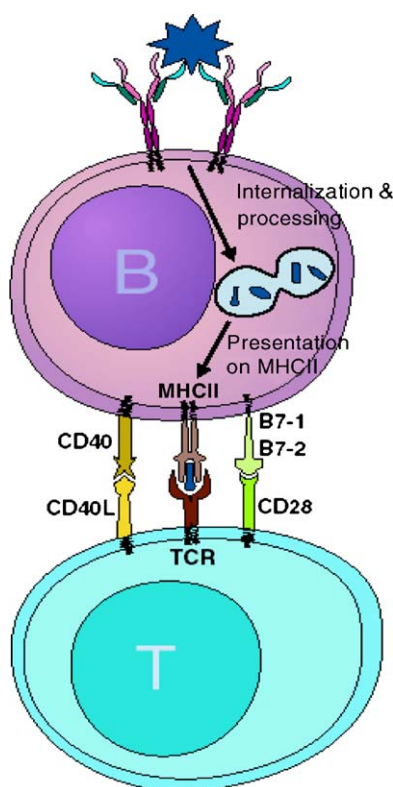


Fig. 2. B-cell-mediated antigen presentation. Antigen binding to B cells expressing allergen-specific IgE on their surface stimulates expression of the co-stimulatory molecules B7-1 and B7-2. Internalization and presentation of allergen-derived peptides on the MHC class II context to T helper cells results in T-cell activation and induction of CD40 ligand. CD40/CD40L interaction induces B-cell proliferation. Because new IgE-secreting plasma cells go through mIgE-expressing B-cell stages during differentiation, CD40/CD40L-mediated B-cell proliferation is an efficient way to stimulate allergen-specific serum IgE production. (*Adapted from* Inführ S, Cramer R, Lamers R, et al. Molecular and cellular targets of anti-IgE antibodies. *Allergy* 2005;60:981; with permission.)

safe allergy vaccines represents one of the most important challenges in allergy and asthma research.

Therapeutic approaches based on recombinant allergens

Recombinant DNA technology enables allergens to be produced with high quality in terms of purity, batch-to-batch consistency, composition, and dosage, satisfying the requirements for therapeutic applications in humans. Theoretically, there is also the possibility of formulating personalized vaccines including only allergens to which the patient is sensitized to, avoiding the risk of inducing new sensitizations during the time course of the therapy [57]. Moreover, the same

technology allows the generation of allergens with reduced IgE-binding capacity [58–60], the production of multimeres [61], the fusion of vaccines containing different allergens [62] or epitopes [63], and the creation of unfolded recombinant molecules voided of IgE-binding epitopes [64,65]. Currently, all proposed concepts for improving SIT based on recombinant allergens remain highly speculative. To date, only two clinical studies involving vaccination with rBet v 1, the major birch pollen allergen, and a mixture of five recombinant *Phleum pratense* allergens are available from the literature [66,67]. Both studies show that recombinant allergen vaccines can be effective and safe, at least for the treatment of allergic rhinitis. Additionally, peptide-based immunotherapy using short synthetic peptides derived from Api m 1, the major bee venom allergen [68], and the major cat allergen Fel d 1 [69] has been clinically evaluated.

Although these studies indicate the possibility of using peptides containing the major T-cell epitopes of allergens for successful immunotherapy, the approach requires prior identification of the T-cell epitopes to be able to produce the synthetic peptides required for treatment. In view of the complexity of the allergen repertoire, it seems questionable that such an approach will be feasible on a large scale.

SIT is the only treatment available for allergic diseases that can cure patients, and recombinant allergens are suitable reagents that can induce long-lasting protection against the natural offending allergens. Open questions regarding successful applications of recombinant allergens for SIT are less related to scientific issues and more to financial and regulatory aspects. Because the regulatory requirements include cGMP material for human use, with the costs of production of vaccines based on a hundred different recombinant allergens, the therapy concept based on these substances is unlikely to be financially affordable. Only in a few exceptions, such as birch pollen or cat allergy, could one expect that a single allergen or a mixture of a few molecular structures would be sufficient to treat the majority of patients.

The most prominent problem in SIT is the dropout of patients in concomitance with the long treatment period required to obtain protection. Classical SIT requires approximately 30 to 80 injections with the respective allergen over 3 to 5 years, and at least 1 hour of medical supervision is required after every allergen injection. Although SIT from a medical perspective is clearly the best treatment for an allergy, few patients can afford or are willing to invest the time for 30 to 80 physician visits lasting more than 1 hour over several years. Patients' compliance for immunotherapy is likely to increase if the vaccination procedures can be drastically shortened. Research to enhance the efficiency of allergy vaccines is focused mainly on improving the adjuvant or the type and form of the antigen used, although some activities are devoted to studying the effects of the route of administration on therapy success. Allergy vaccines are generally administered subcutaneously or intradermally, from where they must reach secondary lymphatic organs to induce an immune response. Recent studies show that direct injection of naked DNA vaccines [70], peptide vaccines [71], and adjuvant [72] into peripheral lymph nodes dramatically enhances the immunogenicity of

the substances. In view of the fact that even large amounts of immunogenic antigens can be ignored by the immune system, as long as they remain outside organized lymphoid tissue [73], these results are not unexpected. The first clinical evaluations of the direct injection of bee venom extract and grass pollen extract into the lymph nodes of correspondingly sensitized patients have shown that three injections scattered over a period of 1 month are sufficient to confer a long-lasting protection against allergen re-exposure (T. Kündig, personal communication, 2006). If confirmed in a large number of patients, these results should have important implications for the clinical immunotherapy of allergic diseases.

Summary

The first two reports on SIT for birch and grass pollen allergy based on recombinant allergens show the feasibility of the approach. Recombinant allergen vaccines can be an effective and safe treatment to ameliorate the symptoms of allergic rhinitis and probably other forms of allergies including allergic asthma. Allergen repertoires can be cloned and characterized in a short time, and each single allergen can be produced at reasonable yields and of a quality satisfying the standards for applications in humans. Nevertheless, the two reports to date on allergen-specific SIT are based on procedures similar to those used in conventional SIT, which spans a long treatment period. To become more attractive, SIT needs to be optimized in terms of dosage, component-resolved composition according to the sensitization pattern of single individuals, and the number of injections required to achieve protection. In an optimal scenario, allergen vaccines would be effective after the administration of a few injections like in conventional vaccinations. The achievement of this goal would substantially decrease the costs related to SIT, increase the patient's compliance, and, consequently, result in a strong reduction of the dropouts associated with classical long-term SIT.

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