We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 12

From Molecular Cloning to Vaccine Development for Allergic Diseases

José Cantillo and Leonardo Puerta

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/52821

1. Introduction

Allergic diseases are manifested in susceptible individual by exposure to proteins named allergens that induce an immune response mediated by IgE antibody. Numerous allergens from different sources such as plants, insects, mites and mammals have been obtained as recombinant molecules by molecular cloning. These types of molecules have shown molecular, functional and immunological properties similar to the corresponding natural allergens and, therefore, could be used for in vitro and in vivo diagnosis test of allergy. An important step was done with the development of variants of allergens with reduced allergenicity and preserved immunogenicity, which paved the way toward its rational use in allergen specific immunotherapy to treat allergies. Few of the allergens cloned have been developed to a stage at which they are suitable for use in clinical studies. However, today the academic and scientific communities note a broad and important activity to offer in the near future preparations with enhanced clinical efficacy and safety. In this work, basic aspects and experimental and clinical results of this process are presented.

2. Progress in the molecular cloning and production of allergens

The molecular cloning has provided a practical and efficient way to obtain highly purified molecules for different purposes; in the biomedical sciences this is evident by the increasing amount of biological products, obtained by recombinant DNA technology, which are commercially available for diagnosis and treatment of different diseases, as well as the wide variety of reagents for basic research. The era of molecular cloning of allergen molecules was initiated in 1988 with the report of a cDNA clone coding for the allergen Der p 1 isolated from a cDNA

INTECH
open science | open minds

© 2013 Cantillo and Puerta; licensee InTech. This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

library of the house dust mite *Dermatophagoides pteronyssinus,* screened with rabbit anti -Der p 1 antiserum [1, 2]. Latter, Tovey, E.R *et al.* [3], using sera from allergic individuals for screening a mite cDNA library also isolated a clone of Der p 1. This strategy was useful to explore the whole spectrum of IgE binding proteins in a natural source and to isolate positive clones to express the molecules [4, 5]. The development and optimization of technology based on the polymerase chain reaction (PCR), have given an important impulse to cloning and identification of new allergens. PCR can be applied to screen cDNA library and amplify specific clones, or to obtain by RT- PCR the nucleotide sequence coding for specific allergens and then cloning in an appropriate vector for expression [6-10]. The numerous nucleotide sequences of allergens reported in data bank have facilitated the isolation of new allergens from RNA material using PCR technology, avoiding the construction of cDNA library and the use of sera from allergic subjects for screening, which is time consuming [11-14]. An expressed sequence tagging (EST) approach was applied to obtaining a large sampling and overview of expressed genomes of several mite species [15], the EST approach involved the partial sequencing of random clones selected from cDNA libraries, allowing the identification of allergens with homology to genes from more distantly related species or even across taxonomic kingdoms.

The bacteria *E. coli* is the preferred expression system used for the production of recombinant allergens, most of the house dust mite allergens have been expressed in this system with success, allowing the molecular characterization [4, 5, 9, 10, 16-18]. The use of *E. coli* may result in non-functional products expressed in inclusion bodies, and without the post-translational modifications necessaries for their appropriate folding and biologic functions [19]. However, by genetic engineering modified strains of this bacteria and novel expression vectors have been obtained, which allow expression of heterologous protein in soluble form with functional properties and high yield; Origami, Rosetta or BL21(DE3)-CodonPlus-pRIL and Rosetta-gami are strains commercially available for obtain recombinants with some pos-translational modifications [20]. In these *E. coli* strains the expression of allergens from the pollen *Artemisia vulgaris* (Art v 3), the peanut (Ara h 2) and the beta-lactoglobulin from bovine have been obtained in higher yield and solubility, and with structural and immunological properties comparable to native allergens [21-23]. The GST tag used in the expression of the first recombinant allergens have been replace for His x6 tag, which is shorter, the recombinant can be analyzed without removing the tag due to the negligible effect on the properties of the molecule, and several efficient purification systems are commercially available.

The eukaryotic expression system have the capacity of performing many of the post-translational modifications including signal sequences, disulfide bond formation, and addition of lipid and carbohydrates. A variety of eukaryotic expression systems like yeast, insect cells, mammalian cells and plants are available. The yeast *P. pastoris* is easy to manipulate and frequently used to express recombinant molecules with all the characteristics of their natural counterparts, with a yield about 10 to 100 times higher than *E. coli* [24, 25]. Several recombinant allergens have been obtained by expression in this yeast and their biologic properties demonstrated by different methods, this system have resulted especially practical when post-translational modifications or biochemical activity exist [26-29]. The human cells have been used to obtain the *Phleum pretense* allergen, Phl p 5, as a secreted or membrane-anchored

http://dx.doi.org/10.5772/52821

protein and showed to be biologically active, with capacity to bind human IgE, to induce mediator release from basophiles and to stimulate T cell proliferation [30]. A large percentage of allergens are from plants, thus the plant-based expression systems are ideal for the production of certain recombinant allergens, which could have problems such as incorrect processing, incorrect folding and insolubility when expressed in bacteria or other non-plant systems. Thaumatin or thaumatin-like proteins, only when expressed in *Nicotiana benthamiana* result in fully IgE-reactive proteins [31]. Interesting, expression in plants offers the opportunity for oral delivery of recombinant allergens of non- plant origin as a therapeutic approach for mucosal immunization for treating allergic diseases. Oral treatment of mice with squash extracts containing virus-expressed Der p 5 allergen caused inhibition of both allergen-specific IgE synthesis and airway inflammation [32], this plant-based edible vaccines is very promising.

3. Current vaccines for allergic diseases

Allergies are inflammatory diseases characterized by a Th2 biased response induced in atopic individuals for exposure to allergens. The Th2 response is also induced by helminthes, which occur in an environment characterized by the presence of IL-4, IL-5 and IL-13. Nuocytes [33, 34], basophiles [35] and type 2 multi-potent progenitor cells [36] seem to be an important source of this cytokines and necessary for the development of allergic response. Allergen-specific IgE antibodies produced by B cells bind to Fc epsilon receptor 1 (FcεRI) on basophiles or mast cells, sensitizing them. After consecutive exposure, allergen binds to IgE on these cells leading to the release of inflammatory mediators of immediate-type symptoms of allergic diseases and paves the way for late-phase inflammatory responses caused by basophiles, eosinophils and T cells. Allergen specific Th1, Th9, Th17 and Treg cells are also produced in this process [37, 38].

Allergen-specific immunotherapy (SIT) is the only curative and specific approach for treatment of allergies [39, 40]. The current SIT consists of gradual administration of increasing amounts of allergenic extract with the aim to avoid allergic symptoms associated to the exposition. The induction of allergen tolerance is the essential immunological mechanisms of SIT, and involve allergen-specific memory T and B-cell that lead to immune tolerance characterized by a specific noninflammatory reactivity to a given allergen and prevention of new sensitizations and progression of allergic disease. During the immunotherapy, different regulatory and effectors components of the immune system are involved (Figure 1). Allergen tolerance is characterized by the generation of two subgroups of Treg cells: $FOXP3^+$ $CD4^+$ $CD25^+$ Treg cells and inducible Treg cells [41]. T-regulatory type 1 (Tr1) cells have shown to play a major role in allergen tolerance induced by SIT [42, 43]. The immunosuppressor mechanism of Treg cells is mediated by the production of high level of anti-inflammatory cytokines IL-10 and TGF-β, although IFN-γ could also be produced [44-46]. The expression of different subtypes of antibodies during SIT is mediated by the activity of regulatory cytokines secreted by Treg cells; IL-10 is a potent suppressor of allergen-specific IgE and simultaneously increases IgG4 production [42]. SIT increase 10 to 100 folds the serum levels of allergen-specific IgG1 and IgG4 [43, 47]. The IgG4 seems to act as a blocking antibody that interacts with the allergen, avoiding interaction of allergen with the IgE [48].

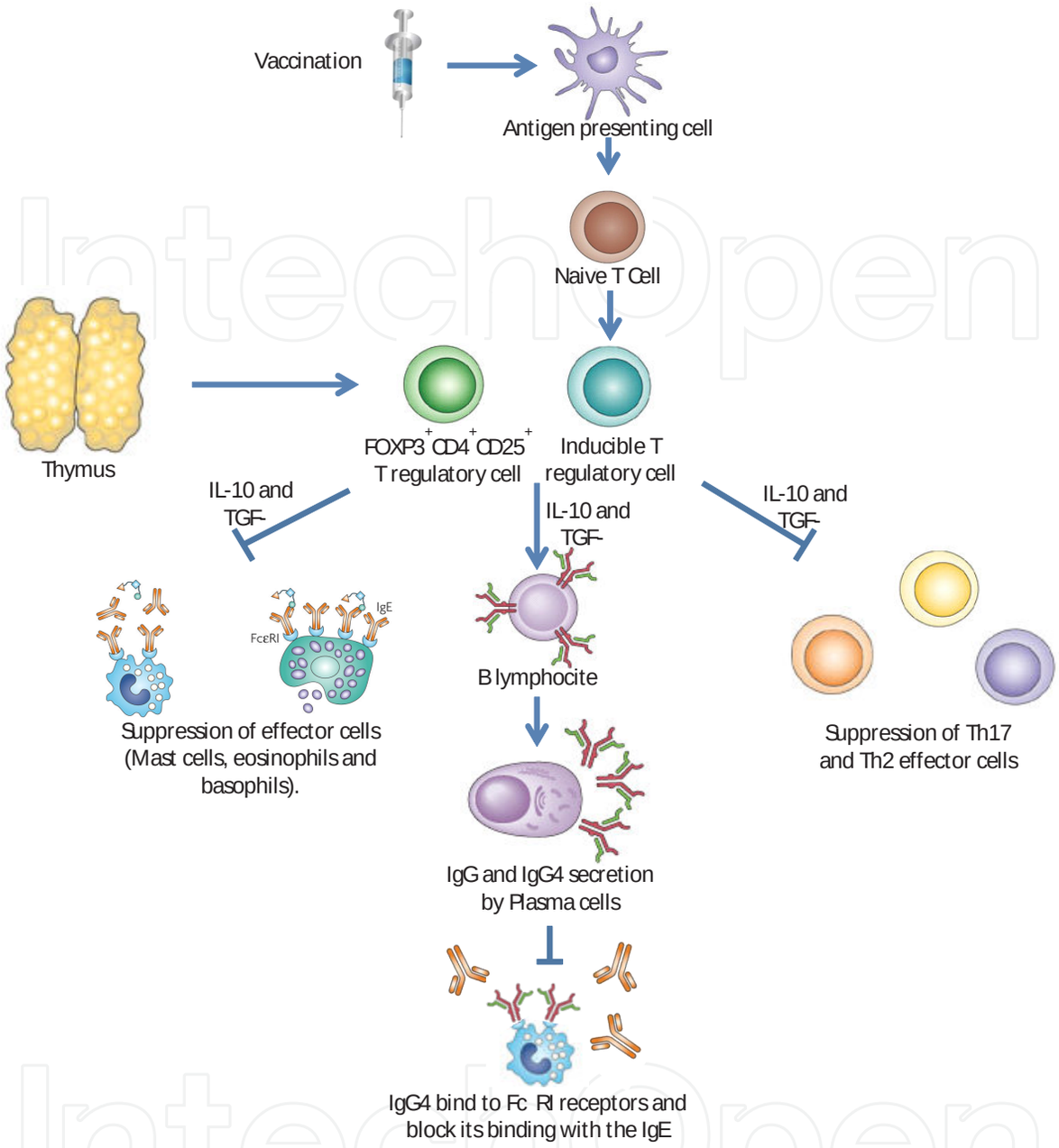

Figure 1. Mechanism of allergen-specific immunotherapy. After vaccination, allergen is taken up by antigen presenting cells leading to the differentiation of naïve T cells to inducible T regulatory cells. These cells with the thymus-derived $FOXP3^{+}CD4^{+}CD25^{+}$ T regulatory cells, suppress allergic response by the following mechanisms. 1. Suppression of mast cells, eosinophils and basophils. 2. Induction of IgG4 antibodies production from B cells that block the binding of allergen with the IgE. 3. Suppression of effector T cells.

Vaccines composed of whole allergenic extract are complex mixtures of known and unknown material, prepared directly from the allergen source, thus containing allergenic and non-allergenic material and being difficult to standardize [49-51]. Some non-allergenic components have been shown to prime a Th2 response [52], which offset the efficacy of this type of vaccines. SIT with allergenic extract induce a variety of side effects ranging from local to systemic which

http://dx.doi.org/10.5772/52821

in some case may be life-threatening [53]. Moreover, in some preparations the important allergens are not well represented or they exhibit poor immunogenicity [51]. Administration of whole allergenic extracts can induce new IgE specificities against allergens present which were not recognized by the patient before treatment [54]. All these facts decrease the efficacy and safety of the current allergen SIT [50]. Therefore, among new approaches to provide a better treatment for allergic diseases is to develop vaccines based on preparations with a well-defined composition, suitable for a good standardization and very low risk of anaphylaxis, here the recombinant allergens or modification of these represent a good option, they show characteristics that could allow to replace advantageously the whole allergenic extracts [55], (Table 1).

Disadvantages of natural allergen extracts
Contain undefined components, some of which may promote allergic responses
Lack or contain low amounts of important allergens
Can be contaminated with unwanted materials or allergens from other sources
Cannot be tailored to the patient's sensitization profile
May induce new sensitizations
Do not suit the international quality standards for vaccines
Cannot be compared between different products or batches
Do not allow the precise monitoring and investigation of mechanisms underlying treatment
Advantages of recombinant allergens
Represent molecules with defined physicochemical and immunologic properties that can be modified to foster advantageous characteristics
Amounts can be easily controlled on the basis of mass units
Potencies and ratios can be exactly adjusted for each molecule
Represents 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 or batches
Allow the precise monitoring and investigation of mechanisms underlying treatment
Can be reproducible modified to suit different treatment strategies

Table 1. Advantages of recombinant allergens over traditional allergen extracts

4. Recombinant allergens for diagnosis and allergen-specific immunotherapy

Recombinant allergens may be obtained with the same structural and immunological properties of its natural equivalent, therefore, the usefulness for diagnosis or immunotherapy is guarantee. These can be expressed in large amounts in *E. coli* or eukaryotic systems at low cost and without contaminants, and manipulating the nucleotide sequence of allergens followed by molecular cloning and protein expression, modified version of allergens that preserve the specific T cell recognition of the natural offending molecule but reduced allergenicity, can be obtained providing a good material for allergen vaccine development.

4.1. Diagnosis of allergy

A more appropriate diagnostic of allergic diseases would be obtained by identification of the particular molecules involved in allergic response, which could be done using purified wild type or recombinant allergens in order to define the sensitization profile of each allergic subject, the concept "Component-resolved diagnosis" was applied to this kind of diagnosis [56], that would allow a "component-resolved immunotherapy", in which only the allergens involved in the sensitization are applied to an allergic subject, avoiding new sensitizations. There are some illustrative examples of the goodness of this future practice: in skin tests with three recombinant cherry allergens, rPru av 1, rPru av 3 and rPru av 4, the diagnosis of allergic population could be obtained with sensitivity similar to that obtained with the allergenic extract [57]. The population allergic to peanut was identified using three recombinant peanut allergens (rAra h 1, rAra h 2 and rAra h 3) [58], in this study and another with celery allergens was demonstrated that recombinant allergens improve the sensitivity of diagnosis compared to allergenic extracts [58, 59]. In allergies with a high compromise of cross-reactivity such as the pollen-related food allergy the power of *in vitro* testing using allergenic extracts is very low [60]. Component resolved diagnosis with recombinant allergens result in excellent sensitivity, when applied to allergy to hazelnut that shows cross-reactivity with pollen allergy. Vespid allergy is characterized by cross-reactivity between hymenoptera species, and it has been established that the true source of sensitization must be defined to ensure the efficacy of venom immunotherapy [61]. Monsalve, *et al.* [62], found that in the Mediterranean regions, a component-resolved diagnosis for wasp allergy could be accurately defined using a mixture of the allergens Ves v 1 and Ves v 5 from Vespula spp, and Pol d 1 and Pol d 5 from *Polistes dominilus*. A combination of these four allergens is enough to differentiate the real causative venom in at least 69% of the population. In allergy with a wide spectrum of sensitization profile as the induced by *Phleum pretense* pollens, the use of recombinants is useful to establish a tailor made immunotherapy approach [63]. A hybrid molecule composed of several segments of a grass pollen allergen showed in skin tests on 32 allergic individuals that with only this molecule all the allergic patients can be identified [64].

The technology of microarrays can be applied to target protein interactions and the serological immune response to antigens [65]. Microarrays are highly useful for detecting all antibodies isotypes and are a powerful tool for component-resolved diagnosis. The primary advantage

of microarrays is that specific IgE to thousands allergens can be assayed in parallel with small amounts of serum, at the same time, much less amount of allergen is required. The advantages of protein microarrays to detect specific-antibodies against multiple targets have been taken to develop component-based diagnosis tools. A microarray based test developed by VBC Genomic and Phadia market as "ISAC" that uses a combination of 103 purified natural and recombinant allergens from 47 species, is available in Europe, however, in the United States it has not yet been approved for use by the US Food and Drug Administration and is available only as a research tool (Available at: http://www.pirllab.com/). One of its potentials lies in the recognition of individual patterns of IgE reactivity to protein families with homologues across plant or animal species [66, 67]. When microarray test for diagnosis of birch and timothy allergy were compared with other *in vitro* tests (Phadia CAP-FEIA and in-house ELISA), a correlation greater than 0.9, with high sensitivity and specificity was obtained [68]. Latex allergy diagnosis is well known to be confounded by a high rate of false positive results when using conventional testing, and positive specific IgE results does not always mirror the clinical situation. A combination of recombinant latex allergens (Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02) on a microarray, was enough to detect individuals allergic to latex with a sensitivity of 80%, and allows discrimination between genuine allergy and sensitization [69]. Recently, a library of 419 overlapping peptides corresponding to the aminoacid sequence of peanut allergens Ara h 1, Ara h 2 and Ara h 3, printed onto glass slides to asses IgE reactivity, was evaluated as a diagnostic tool that could replace the traditional used double-blind, placebo controlled food challenge, that is time consuming, expensive, stressful for the patient and have the risk for potentially life-threatening anaphylactic reaction [70, 71]. Based on the number or peptides that bind IgE and the intensity of the reaction, was possible to distinguish peanut allergic and peanut tolerant individuals with approximately 90% sensitivity and 95% specificity [71].

To evaluate the clinical significance and allergenicity of several recombinant allergens from *B. tropicalis* and *D. pteronyssinus* in asthmatic patients from a tropical environment, IgE level were determined in sera from 90 asthmatic patients and 10 healthy controls. In addition, SPT was performed in a selected group of these patients [72]. Three recombinant allergens Der p 1, Der p 2, and Der p 10 were able to detect 93% of *D. pteronyssinus* allergic subjects. No adverse reactions were observed in the allergic or control subjects who were skin tested. We can conclude that recombinant allergens from *B. tropicalis* and *D. pteronyssinus* are useful for *in vitro* and *in vivo* diagnostic tests of mite allergy diseases.

4.2. Allergen-specific immunotherapy

Allergen SIT with recombinant allergens was proposed when it was demonstrated that these molecules have similar or the same biological properties of their natural counterparts [73, 74], and the necessity of highly purified and well standardized allergens were required for overcome the problems related to difficult standardization and management of the doses observed with the whole allergenic extracts. A study with the recombinant pollen allergen Bet v 1 (rBet v 1) demonstrated that immunotherapy with a single allergen is effective for the specific treatment of allergy [75]. In a multicenter, double-blind, placebo-controlled clinical trial, patients with history of birch pollen–related rhinoconjunctivitis were divided in four

groups and treated for two years with rBet v 1, natural birch pollen extract, natural Bet v 1 (nBet v 1) or placebo, to compare the efficacy of each preparation for allergen-specific immunotherapy. Treatment with rBet v 1 reduced symptoms of rhinoconjunctivitis and skin reactivity induced by birch pollen, and showed to be safety without serious adverse events. In contrast, one adverse event appears in the group treated with nBet v 1. Clinical improvement and reduction of sensitivity were accompanied with marked increase in Bet v 1-specific IgG1, IgG2 and IgG4 levels, which were higher in the rBet v 1-treated group than in nBet v 1-treated group. Importantly, new IgE specificities were induced in 3 patients treated with birch pollen extract, but in none of rBet v 1 or nBet v 1 treated patients.

In a placebo controlled immunotherapy study, a mixture of equimolar concentration of five *Phleum pretense* allergens (Phl p 1, Phl p 2, Phl p 5a, Phl p 5b and Phl p 6) was administered via subcutaneous for 18 months in patients with grass pollen–induced allergic rhinitis, to determining efficacy and safety [43]. The immunotherapy showed a 36.5% lower median average symptom score for active treatment compared with placebo and reduction in the need for medication. By the first and second pollen season, improvement in quality of life scores was present in the patients receiving active treatment. Active treatment induced IgG1 concentrations approximately 60-fold, peaking during the rusty 12 months of the study and IgG4 levels showed a 4000 fold increase by the end of treatment. In contrast, after immunotherapy IgE levels in the active treated group were significantly lower than placebo group. Only about 1% of recombinant grass allergen injections led to systemic reactions. This was the first clinical study of immunotherapy with a cocktail of 5 recombinant grass pollen allergens that showed its clinical efficacy, good tolerance and strong induction of allergen specific IgG antibody response.

5. Approaches for an immunotherapy of allergy based on modified recombinant allergens

Several *in vitro* and *in vivo* assays indicate that allergy vaccines based on recombinant allergens might have provide a safe and efficacious immunotherapy. However, recombinants with the same amino acid sequence and similar allergenic activity as the natural allergens can elicit IgE-mediated side effects, which are a major risk during allergen-specific immunotherapy. To overcome this problem different approach have been designed to obtain molecules without or reduced IgE reactivity [76]. Recombinant DNA technology has allowed the rational design and production of well-defined modified allergens for this purpose. Hypo-allergens are molecules derived from wild type allergen which exhibits reduced capacity to react with IgE antibodies and low ability to induce IgE-mediated mast cells or basophile degranulation. They are designed to reduce the risk of anaphylaxis during the course of immunotherapy, are molecules with conserved T-cell epitopes that could be recognized by specific T lymphocytes to induce a protective response against wild-type allergen.

Some allergens are present in the nature as a mix of several isoforms with high structural homology but different IgE reactivity. The production by molecular cloning of natural isoforms

with low IgE reactivity has been used to propose anti-allergy vaccines. Bet v 1.0401 and Bet v 1.1001 are isoforms that have lower IgE reactivity compared to the Bet v 1.0101 [77, 78]. Antibody response against Bet v 1.0401 is IgG4-specific and has low capacity to induce basophile degranulation [79].

Hypo-allergens obtained by site-directed mutagenesis

The availability of multiple clones of recombinant allergens has facilitated the implementation of site directed mutagenesis to obtain modified allergens for a better immunotherapy. There are several examples of this approach that illustrate the potential use for the development of new vaccines. Mouse allergic individuals are sensitized mainly against the major allergen Mus m 1 a urinary protein belonging to the lipocalin superfamily which have typicall β-barrel fold, that can be modified by mutation in the Tyr 120 residue, [80, 81]. Two hypo-allergenic variants of this allergen; mutants 1Y120A, and Mus m 1-Y120F were expressed in *P. pastoris* with a modified fold [82]. The mutants showed low capacity to react with IgE from allergic individuals and induced lower basophil degranulation than those induced by Mus m 1. In lymphoproliferation assays, using cells from mouse allergic individuals the mutants induced similar lymphoproliferation to that induced by Mus m 1. In other study three variants of an allergen from Artemisia (Art v 1), C22S, C47S and C49S [83], showed low IgE reactivity and mediator release from RBL cells. In addition, the variants C49S and C22S induced significantly higher T cell proliferative response in Artemisia allergic patients compared to the obtained with rArt v 1, suggesting a potential utility for the immunotherapy of population allergic to Artemisia. Using a different approach; mutations in residues involved in IgE binding but maintaining the 3D structure of the natural allergen, Spangforth et al. showed that mutants Gln45-Ser and Pro108-Gly of allergen Bet v 1 displayed lower IgE reactivity and induced synthesis of IgG antibodies that block the IgE reactivity against the natural Bet v1 [84]. Unlike the above mentioned studies. The x-ray crystallography structures of the mutants were similar to the natural allergen, indicating that the reduced IgE reactivity is not mediated by an inadequate folding.

Hybrid proteins

Hybrid proteins are structures composed by two or more allergens or short portions of them in only one molecule, in this way new interaction and bonds are generated, which may alter the 3D structure and B epitopes characteristic of natural allergens. Decreasing the capacity of IgE binding and mast cell degranulation. However, if these proteins conserve the T cell epitopes, they could induce a protective response after allergen challenge. A single molecule composed by different allergen polypeptides might reduce the number of molecules to be included in the vaccine. Furthermore, hybrid molecules consisting of several copies of homologous allergens or immunologically unrelated allergens could be used for the allergy treatment in the patients who are sensitized to several allergens.

T. P. King, who constructed a molecule composed by two allergens from insect and demonstrated *in vitro* and mice models studies its anti-allergenic properties [85], pioneered the design of hybrid proteins for allergen immunotherapy. Gonzales-Rioja *et al*. [86] obtained by PCR-based engineering a molecule composed by two allergens from the pollen *Parietaria judaica*

(Par j 1 and Par j 2) and demonstrated an important reduction of the IgE binding capacity by skin prick test. Linhart *et al*. [87], constructed by PCR-based recombination, a nucleotide sequence coding for principal allergens of timothy grass, Phl p 1, Phl p 2, Phl p 5 and Phl p 6. The hybrid protein induced T cell proliferation similar to the equimolar mix of individual allergens, the lymphocytes secreted regulatory and Th1 cytokines, IL-10 and IFN-γ, showing capacity to induce immune deviation to a protective profile. In an allergic mouse model, exposure to hybrid molecule induced the production of IgG that blocked mast degranulation. However, this molecule was also capable to bind IgE from allergic individuals, and to induce basophile degranulation, and high percentage of individuals allergic to timothy grass were identified using this molecule, suggesting a potential as a diagnosis reagent.

The utility of hybrid proteins for the immunotherapy of house dust mite allergy have been studied by Asturias, *et al*. [88], who designed two hybrid proteins composed by *D. pteronyssinus* allergens Der p 1 and Der p 2. The QM1 structure was composed of almost the whole sequence of both allergens; a Der p 2-fragment from residues 5 to 123 at the N-terminus, introducing point mutations on cysteine 8 and 119 to serine to avoid the formation of a disulphide bridge, and a Der p 1-fragment from residues 4 to 222 at the C-terminus were joined. The QM2 structure, was composed with the residues 1 to 73 and 74 to 129 of Der p 2, linked to residues 5 to 222 of Der p 1. Western-blot assays with a serum pool from house dust mite allergic patients showed decrease IgE reactivity of QM1, while QM2 showed no detectable IgE binding capacity. The hypoallergenic properties of both hybrids were demonstrated by skin tests. In vitro test with sample from allergic patients QM2 induced similar lymphoproliferation that the induced by natural Der p 1 and Der p 2, whereas, QM1 induced higher proliferation. It was demonstrated that antisera raised by immunization of mice with QM1 or QM2 lead to the production of specific antibodies capable of blocking the binding of IgE reactivity to natural allergens.

Recently, a hybrid protein composed of three allergens of *Chenopodium album* pollen, in the order Che a 3-Che a 1-Che a 2, was constructed by using overlapping extension polymerase chain reaction, expressed in *E. coli* BL21-CodonPlus(DE3)-RIL, to obtain a 46 kDa protein. Sera from allergic patients showed lower IgE binding affinity to the hybrid molecule than the mixture of recombinant allergens and the *C. album* pollen extract. Most of the allergic patients showed positive skin test to a mixture of the three allergens, however, when tested with the hybrid molecule allergic patients showed negative test or highly reduced weal area compared to the mixture or to the pollen extract [89].

Mosaic proteins

Mosaic proteins are constituted by different segments of the same allergen, in different order as they are present in the native molecule, such re-arrange generate new intra-molecular interactions that alter B cell epitopes. A mosaic protein called P1m constructed with four segments of the pollen allergen Phl p 1, showed lower IgE reactivity compared to the natural allergen and was unable to induce histamine release from basophiles of allergic individuals. However, this molecule conserved capacity to induce the proliferation of PBMCs. Immunized rabbits expressed IgG antibodies that blocked the binding of Phl p 1 to the IgE and inhibit histamine release from basophiles obtained from allergic individuals [90]. Other mosaic

protein constructed with segments derived from Phl p 2 reassembled in altered order and expressed as a trimer showed absence of IgE reactivity with sera from allergic patients. Basophile activation and skin prick tests, showed reduction of the allergenicity of this molecule compared to recombinant Phl p 2. Furthermore, IgG antibodies produced by immunized mice were able to inhibit the binding of recombinant Phl p 2 to the IgE from allergic subjects [91]. Mosaic proteins have been studied as a potential vaccine for immunotherapy of birch allergy [92] and house dust mite allergy [93]. A mosaic protein composed of reorganized segments of Bet v 1 preserved the specific T cell epitopes and showed approximately 100-fold reduced allergenic activity compared with recombinant Bet v 1 [94, 95] and induced specific IgG antibodies inhibitors of IgE reactivity to Bet v1 of sera from patients with pollen allergy [96]. The mosaic protein exhibited none IgE reactivity and lower basophile activation. Furthermore, immunization with Bet v 1 derivatives induced IgG antibodies that recognized Bet v 1 and inhibited IgE binding to Bet v 1 [92].

Fragments of allergens or modification of these, might be poorly immunogenic because they don´t have enough T cell epitopes capable to stimulate a protective immune response. An increase of immunogenicity can be obtained when proteins are made as oligomers which enhance the number of T cell epitopes in the molecule. It has been observed that immunogenicity of Bet v1 increase when obtained as oligomer [97, 98]. By dot-blot analysis and lymphoproliferative responses in PBMCs from birch pollen allergic patients, trimers of re-organized segments of Bet v1 had lower capacity to bind IgE and enhanced capacity to stimulate lymphoproliferation. The CD203c expression analysis showed reduced allergenicity of these oligomers, and when administrated to mice in an immunization scheme, induced the production of high titer of IgG1 antibody, that inhibited human IgE binding to wild type Bet v 1 [99].

5.1. Molecules to target specific compartments or receptors

Targeting allergens to endoplasmic reticulum

It has been suggested that administration of higher allergen doses enhances the efficacy of immunotherapy [100]. However, administration of high doses increases the risk of anaphylaxis. One approach to overcome this problem is to deliver high doses of allergen to B and T cells directly, thus providing higher effective doses to stimulate a protective response, and avoiding the interaction of allergen with IgE antibodies [101]. An allergen vaccine for cat allergy composed of the major cat allergen Fel d 1 fused to the HIV-derived translocation peptide TAT was designed to mediate cytoplasmic uptake of extracellular proteins [102, 103]. Un modified version of this approach, denominated Modular Antigen Translocation (MAT) technology have been developed [104, 105], which consists of allergen fused to a peptide, to direct them to the cytosol, and a truncated human invariant chain (Ii), to target the protein to MHC class II heterodimers assembled in the endoplasmic reticulum and thus circumventing phagosomal uptake and degradation. The allergens Asp f 1, Der p 1, Bet v 1, PLA2 and Fel d 1 fused to MAT, induced lymphoproliferation of PBMCs stimulated *ex vivo* with low allergen doses, induced the secretion of Th1 type cytokines and IL-10, and inhibit the production of Th2 cytokines [105]. The cat allergen Fel d 1 fused to MAT (MAT- Fel d 1) when administered directly to the inguinal lymph nodes of allergic mice, showed capacity to stimulate the

production of high levels of IFN-γ and reduced levels of IL-4, compared to unmodified Fel d 1. Immunized mice expressed higher levels of IgG2a and showed protection against the challenge of high doses of allergenic extract. Furthermore, MAT-Fel d 1 produced 100-fold less degranulation and histamine release from basophiles compared to unmodified Fel d 1 [106].

Targeting allergens to receptors on dendritic cells

Dendritic cells (DCs) play an important role in the initiation and maintenance of T cell response to allergens. Its role in the type of T cell response generated can be influenced by the maturation state, while mature DCs induce effector T cell responses characterized by Th1 or Th2 response [107], immature or semi-mature DCs are tolerogenic and have the ability to induce Tregs [108]. DCs express an array of Fc receptors which have the capacity to enhance allergen uptake through internalization of allergen/antibody receptors complexes. When stimulated with allergen, DCs express FcεRI, and activated a signal-transducing cascade involving immunoreceptor tyrosine-based activation motif (ITAM), which result in increased production of proinflammatory cytokines and chemokines, the induction of robust proliferation of allergen-specific T cells and the development of allergic symptoms [109]. DCs also express the receptor FcγRIIb that contains immunoreceptor tyrosine-based inhibition motif (ITIM) which induces inhibitory signaling events. This receptor can co-aggregate with FcεRI that activating a signaling cascade that culminates in inhibition of FcεRI signaling. Under these assumptions, Zhu, D. *et al*. [110] designed a fusion molecule called GFD composed by a human IgG Fc fragment linked to the allergen Fel d 1 by a flexible linker, with the aim to crosslink FcγRIIb and FcεRI-bounded to the cat specific-IgE. In transgenic mice expressing human FcγRIIb and FcεRI, sensitized with high doses of Fel d 1 specific-IgE and treated with several doses of GFD, the challenge with Fel d 1 didn´t cause mast cell degranulation. A scheme of immunotherapy with high doses of GFD resulted in the inhibition of allergic response against Fel d 1, pulmonary inflammation and skin reactivity in sensitized animals. Treated mice expressed IgG1 antibodies that blocked the binding of IgE to Fel d 1. When applied to mice sensitized to Fel d 1 in a scheme of rush immunotherapy, GFD blocked acute systemic allergic reaction, mast cell degranulation, bronquial hyper-reactivity and pulmonary inflammation [111]. Recently, a fusion protein composed of Fcγ chain and the *Dermatophagoides farina*e allergen, Der f 2, was obtained and tested in a Der f 2 allergic murine model [112]. After treatment with the fusion molecule, the levels of specific IgE to Der f 2, histamine and pro-inflammatory cytokines were lowered in the Fcγ-Der f 2 treated allergic mice, compared to saline-treated allergic mice. These results suggest that specific targeting of allergens to Fcγ receptors could be used as a strategy in the development of antigen-specific immunotherapy for human allergic diseases.

A different molecular design was applied to target allergens to CD64 receptor on antigen presenting cells; a fusion protein (H22-Fel d 1) composed by Fel d 1 linked to the variable region of a monoclonal antibody anti-CD64 was designed to stimulate receptor internalization [113]. Flow cytometry analysis showed that H22-Fel d 1 binds to CD64 and reacted with IgE and IgG with similar affinity compared to native allergen. *In vitro* assays demonstrated that the fusion molecule stimulates the proliferation of T lymphocytes derived from allergic individuals and the secretion of IL-5, IL-10 and IFN-γ [114]. Although H22-Fel d 1 is responsible of a positive effect that could result in a protective response against allergen challenge, it also stimulated

http://dx.doi.org/10.5772/52821

Th2 cytokines, in a mechanism in which the thymic stromal lymphopoietin (TSLP) cytokine seems to be involved. This cytokine was shown to maintain and polarize circulating Th2 central memory cells, including allergen-specific T cells [115]. Therefore, the usefulness of this kind of preparation for allergy immunotherapy deserves further evaluation.

6. Insect sting allergy

Insect sting allergy are frequently caused by insect stings of the Apidae family (honeybees and bumblebees), those from the Vespidae family (Vespula, Dolichovespula, Vespa and Polistes genera) and, in some regions, also of the Formicidae family (ants). The sting can induce local or systemic IgE-mediated hypersensitivity reactions that can be fatal [116]. Prevalence of systemic reactions caused by insect stings are reported from 0,3% to 7,5% in the United States and Europe [117, 118]. Up to one fifth of these subjects will eventually experience severe life-threatening reactions. Hymenoptera venoms contain protein allergens, as well as non-allergenic components, including toxins, vasoactive amines, acetylcholine, and kinins. Among the multiple allergens in Hymenoptera venoms, two allergens are importan, the phospholipase A2 from of honey bee (*Apis mellifera*) (Api m 1), and of the vespid venoms antigen 5 from *Vespula vulgaris* (common wasp) denominated Ves v 5.

Several studies have demonstrated that immunotherapy for vespid allergy with venom extracts is clinically effective and improve the quality of life and allergic symptoms. This improvement is correlated to a significant decrease of total IgE levels, and increase in specific IgG and IgG4 levels [119]. However, severe and life-threatening anaphylactic side effects may be induced after the administration of crude allergen extracts [120].

One of the first attempts to obtain safer methods for immunotherapy of insect allergies was made with allergen-derived peptides, containing T-cell epitopes. Peptides derived from the bee allergen Api m 1, were applied to allergic individuals in different immunotherapy schemes. *In* vitro and clinical phase trials showed that T cells from such patients showed marked responsiveness to Api m 1 after long term treatment, a shift in the pattern of cytokine secretion form a Th0 to a Th1 profile and increase in specific IgG4 levels [121-123].

The use of recombinant venom allergens for allergen specific immunotherapy has been analyzed in animal models. Intranasal administration of the recombinant allergen from wasp venom, rVes v 5, to mice prior to sensitization with natural allergens lead to a significant reduction of the allergic reaction, reduction of specific IgE and IgG2a levels, increase of mRNA levels of IL-10 and TGF-β. Pretreatment with the whole venom was less effective and caused toxic side reactions, suggesting a favorable use of the recombinant protein [124]. Hybrid proteins composed by allergens from bee venom have shown anti-allergenic properties in *in vitro* and animal models [85]. A fusion protein composed of the two major bee venom allergens Api m 1 and Api m 2 called Api m [1/2], showed reduced IgE reactivity of Api m [1/2] compared with native allergens [125]. By the other hand, basophil degranulation and skin tests showed that this fusion protein have hypo-allergenic properties. When applied subcutaneously, mice

showed reduced specific IgE, IgG and IgG2 serum levels; demonstrating that such protein represents a potential candidate for specific immunotherapy.

Naturally occurring variants of insect allergens could be also useful for specific immunotherapy. For example, the sting of *Polybia scutellaris*, a South American wasp, does not cause allergic symptoms, however it has been proven that its venom contain Antigen 5 (Poly s 5), an analogue of the allergen Pol s 5 [126, 127]. In mice, Poly s 5 induced IgG antibodies that cross react with Pol a 5, but induced only minimal amounts of IgE and was poor inducer of basophil-mediator release. Moreover, Poly s 5-specific serum showed a specific protective activity and was able to inhibit Pol a 5-induced basophil degranulation [128].

Despite the promising results observed with recombinant and modified allergens in *in vitro* and *in vivo* studies, more clinical phase studies need to be performed to demonstrate their applicability for the allergen specific immunotherapy of insect allergy.

7. Recombinant allergy vaccines in clinical phase trials

Clinical trials with recombinant wild type allergens, and modified allergens have been performed (Table 2). The first studies of allergen SIT with purified molecules were done with peptides containing T cell epitopes either from the cat allergen Fel d 1 or from bee-venom-derived phospholipase, administered without adjuvant [122, 123, 129-135]. Such peptides were characterized by its low or none IgE binding capacity. However, they induced late phase systemic side effects in different grades depending in the dose and route of administration [129, 132, 134, 135]. Therapy with T cell peptides does not seem to influence IgE-mediated allergic reactions, in fact, the majority of studies didn't find evidence of changes in IgE levels or IgE-mediated allergic inflammation furthermore, no induction of IgG response was noted.

Allergic patients under immunotherapy with hypoallergenic preparations of the major birch pollen allergen Bet v 1 adsorbed to aluminum hydroxide as a pre-seasonal treatment for birch pollen allergy in a clinical trial, expressed high levels of IgG1, IgG2 and IgG4 antibodies directed against Bet v 1. These IgG antibodies blocked allergen-induced basophile degranulation and were associated with the ability of patients to tolerate higher allergen concentrations in nasal provocation tests [136]. Immunotherapy with wild-type recombinant Bet v 1 has also been examined for tablet-based sublingual immunotherapy in a phase II, multicenter, double-blind, placebo-controlled, however, this study is still on course and only have been reported good tolerability of the preparation with no serious adverse events and most side effects observed locally [137].

In a clinical trial, a group of patients with grass pollen allergy was treated with a combination of the major grass pollen allergens (Phl p 1, Phl p 2, Phl p 5a, Phl p 5b and Phl p 6) or with placebo for subcutaneous immunotherapy [43]. Patients treated with the recombinants improve their symptom medication score and had high IgG antibodies levels against natural grass pollen allergens. Several studies of immunotherapy with these mixed allergens have been performed and registered in the National Institutes of Health Clinical trial database (Table

2). Recently, the immunomodulatory properties of MAT-Fel d 1 was studied in a phase I/IIa clinical study [138]. In a randomized double blind trial, intralymphatic immunotherapy (ILIT) with MAT-Fel d 1 in alum was compared with placebo, consisting in 3 injections of each preparation for two months. MAT-Fel d 1 caused reduced skin reactions compared to equimolar concentration of nFel d 1 by intradermal injection, which proved practically painless and reduced drug-related adverse effects compared to placebo group. The IgG4 serum levels in MAT-Fel d 1 treated group increased by a factor of 5.66, while IgG1 and IgE levels didn´t change. After treatment, PBMCs from allergic individuals secreted higher levels of IL-10 when challenged with rFel d 1. Immunotherapy with MAT-Fel d 1 showed to be successful because patients increased their tolerance to nasal challenge, skin prick and dermal test, with cat dander extract. Improvement of quality of life of patients treated with MAT-Fel d 1 was maintained 300 days after immunotherapy.

Allergen	Allergen-based vaccine	Rout of administration/Trials	Year	NIH Registration number / Reference
Bet v 1 (Birch pollen allergen)	Bet v 1 trimer	SCIT, DBPC, Phase II	2000	[125]
	Bet v 1 fragments	SCIT, DBPC, Phase II	2000	[125]
	Bet v 1 folding variant	SCIT, OC, Phase II	2002	NCT00266526
		SCIT, DBPC, Phase III	2004	NCT00309062
		SCIT, DBPC, Phase III	2007	NCT00554983
		SCIT, Immunological and histological evaluation	2009	NCT00841516
	Recombinant Bet v 1	SCIT, DBPC, Phase II	2002	NCT00410930
		SLIT, Phase I	2006	NCT00396149
		SLIT, Phase I	2007	NCT00889460
		SLIT, DBPC, Phase II	2008	NCT00901914
Birch pollen and apple allergens	Bet v 1 / Mal d 1	SCIT, DBPC, Phase II	2011	NCT01449786
Phleum pratense allergens	Mix: Phl p 1, Phl p 2, Phl p 5a, Phl p 5b and Phl p 6	SCIT, DBPC, Phase II	2002	
		SCIT, DBPC, Phase III	2004	NCT00309036
		SCIT, DBPC, Phase II	2008	NCT0671268
		SCIT, DBPC, Phase III	2009	NCT01353755
	Phleum pratense peptide fused to carries protein	SCIT, Phase II	2011	NCT01445002
		SCIT, DBPC, Phase IIb	2012 (Initiating)	NCT01538979
Peanut allergens	Modified Ara h 1, Ara h 2, Ara h 3	Rectal	2009	NCT00850668

NCT numbers identify the trials that are registered in the National Institutes of Health Clinical trial database.

DBPC, Double-blind, placebo-controlled; OC, open controlled; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy.

Table 2. Currently ongoing recombinant molecules development for allergen specific immunotherapy.

The National Institutes of Health's clinical trial database contain information about a study that intends to use the recombinant modified peanut allergens Ara h 1, Ara h 2 and Ara h 3 encapsulated in heat/phenol-killed *E.coli*. This phase I study should recruit healthy volunteers to receive four scaling doses of the peanut preparation rectally at weekly intervals. The major allergen of ragweed pollen *Ambrosia artemisifolia*, Amb a 1, was conjugated to CpG oligonucleotides to reduce the allergenic activity of Am b a 1 and to shift the specific Th2 response to a Th1 response, mediated by the binding of CpG with toll-like receptor 9. Allergic individuals treated with the conjugated vaccine showed reduction in eosinophilia and the number of IL-4-producing cells, and increased numbers of IFN-γ-producing cells compared to placebo-treated patients [139]. Furthermore, increase of regulatory T cells infiltration in the nasal mucosa was found after the course of immunotherapy [140].

8. Some considerations for a recombinant based mite allergy vaccine

The prevalence and severity of allergic diseases such as asthma and rhinitis have increased in recent decades [141], and house dust mite allergy is one of the most common allergies worldwide which affect more than 50% of allergic patients [142]. Several house dust mites species co-exist in tropical and subtropical regions, however in these places the species *B. tropicalis* and *D. pteronyssinus* are the most prevalent and a high percentage of allergic population is sensitized to allergens from these two species [143, 144], [72, 145]. Analysis if house dust mite extracts have shown that over 20 different proteins can induce IgE antibodies in allergic populations and several of them show cross-reactivity with allergens from other mite species. Most of them have been obtained and characterized by molecular cloning and its IgE reactivity analyzed [4]. However, it has been suggested that the majority of mite-allergic subjects elicit an IgE response to around five components of allergenic extracts [146, 147], and some of them may be cross-reactive. Therefore, and admixture of few allergens, including those species-specific and cross-reactive, could replace the crude allergenic extract for diagnostic and therapeutic purpose. Several studies indicate that a combination of allergens from group 1 and 2 bind to more than 50% of specific-IgE from allergic population, groups 5 and 7 are next in importance [148-150]. It has been reported from Middle Europe that more than 95% of mite allergic patients were mainly sensitized to Der p 1 and Der p 2, and that diagnostic test containing these allergens plus the highly cross-reactive allergen Der p 10 may improve the diagnostic selection of patients for immunotherapy with *D. pteronyssinus* extracts [151]. Other allergens are important given their cross-reactivity and the role that they play in tropical populations, as the case of group 10 and 12 [16, 152]. Results from our research group suggested that a combination of these allergens from D. pteronyssinus might be sufficient to identify almost all our mite allergic population [72] (Figure 2).

Recent studies with hybrid proteins composed by the most important pollen allergens, have suggested that preparations based on molecules containing the B-epitope spectrum of allergenic extracts could be useful for the diagnosis and allergen-specific immunotherapy [64, 86, 87]. We have engineered several fusion proteins composed by segments of different allergens of *B. tropicalis* and *D. pteronyssinus* with the aim to obtain preparations useful for the

http://dx.doi.org/10.5772/52821

diagnosis and immunotherapy of allergies caused by house dust mites. The coding sequences of each molecule was cloned into expression vectors and then expressed in *E. coli* fused to 6xHis tag for further purification by affinity chromatography. One of these proteins denominated DPx4, consistent of different segments of allergens from *D. pteronyssinus* (Der p 1, Der p 2, Der p 7 and Der p 10), showed a 41% frequency of IgE reactivity in sera from mite allergic patients sensitized to *D. pteronyssinus* and the specific IgE levels against the recombinant were significantly lower than those against the whole allergenic extract from mites. Basophil activation test showed that DPx4 has lower capacity to induce basophile degranulation compared to the allergenic extract. These results suggest that the fusion protein have a hypoallergenic profile, and that is a good candidate for develop a vaccine with potential use for allergen specific immunotherapy of mite allergy [153]. Further *in vitro* studies as well as experiment with animal models are in progress to support this application.

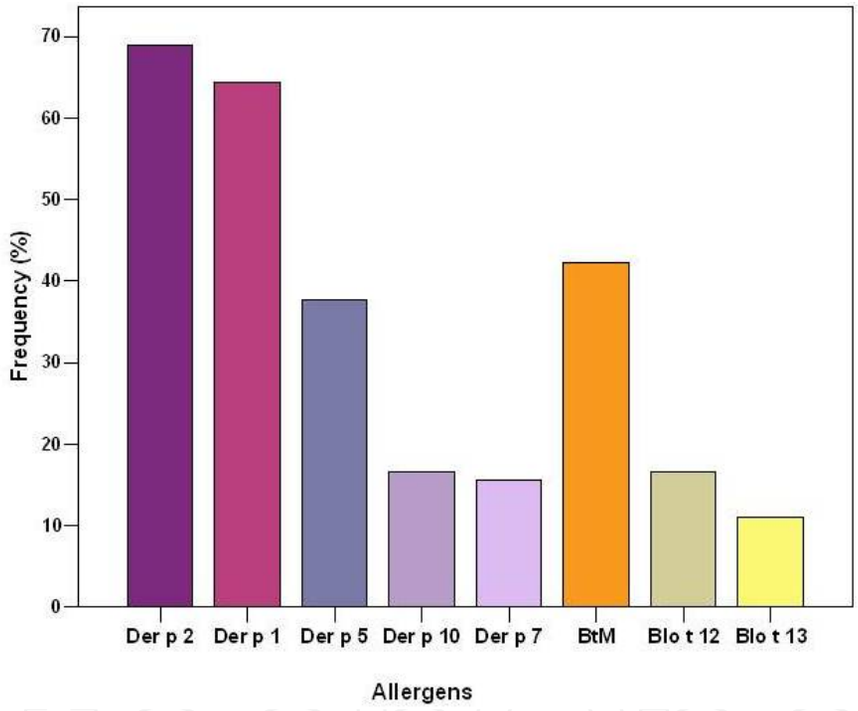

Figure 2. Frequency of IgE reactivity to allergens from *B. tropicalis and D. pteronyssinus* in asthmatic patients (From Ref 72).

9. Conclusions

For several years the allergen-specific immunotherapy has been successfully done with natural allergenic extracts. However, they are complex mixtures difficult to standardize that might cause local or systemic reactions, compromising the patient's life. In the last decades, the molecular cloning applied to the study of allergens has allowed obtaining several recombinant allergens from different sources, and their biological and molecular properties elucidated.

Component based diagnosis and immunotherapy is now possible by the availability of several recombinant allergens, which represents the best approach to achieve the most efficacious diagnosis and treatment of allergies, based on the sensitization profile and of each patient. Vaccines for allergic diseases based on recombinant allergens or modification of these, that could modulate the immune response against natural allergens toward a protective response, have been proposed. Hypoallergenic molecules obtained by molecular cloning, in different versions like hybrid molecules, oligomers, mosaic proteins or variants obtained by site-directed mutagenesis have been developed and studied by in vitro test, animal model and clinical trial in humans, indicating potential beneficial use in the near future. Recombinant allergens coupled to carriers for directing the molecule to specific cells or intracellular compartments, preventing unwanted side effects and increasing the specificity of the immune response have been explored.

The promising results showed by *in vitro* and animal models studies have encouraged the design of clinical phase trials where recombinant allergens have demonstrated their good potential to provide a more efficacious and safe diagnosis and allergen-specific immunotherapy. In the last years, the number of clinical phase trials designed and registered in the National Institutes of Health Clinical trial database is increasing. This tendency suggests that in few years several vaccines based on recombinant allergens could be commercially available in replacement of the traditional allergenic extract.

Acknowledgements

Supported by the Colciencias and University of Cartagena, Colombia. Grant No. 385-2009.

Author details

José Cantillo and Leonardo Puerta*

*Address all correspondence to: lpuertall@yahoo.com

Institute for Immunological Research, University of Cartagena, Colombia

References

[1] Thomas WR, Stewart GA, Simpson RJ, Chua KY, Plozza TM, Dilworth RJ, et al. Cloning and expression of DNA coding for the major house dust mite allergen Der p 1 in Escherichia coli. Int Arch Allergy Appl Immunol 1988;85(1):127-9.

[2] Chua KY, Stewart GA, Thomas WR, Simpson RJ, Dilworth RJ, Plozza TM, et al. Sequence analysis of cDNA coding for a major house dust mite allergen, Der p 1. Homology with cysteine proteases. J Exp Med 1988 Jan 1;167(1):175-82.

[3] Tovey ER, Johnson MC, Roche AL, Cobon GS, Baldo BA. Cloning and sequencing of cDNA expressing a recombinant house dust mite protein that binds human IgE and correspond to an important low molecular weight allergen. J Exp Med 1989;170:1457-62.

[4] Thomas WR, Smith WA, Hales BJ, Mills KL, O'Brien MR. Characterization and immunobiology of house dust mite allergens. Int Arch Allergy Immunol 2002;129:1-18.

[5] Puerta L, Caraballo L, Fernandez-Caldas E, Avjioglu A, Marsh DG, Lockey RF, et al. Nucleotide sequence analysis of a complementary DNA coding for a Blomia tropicalis allergen. J Allergy Clin Immunol 1996 Nov;98(5 Pt 1):932-7.

[6] Cui Y, Zhou Y, Shi W, Ma G, Yang L, Wang Y, et al. Molecular cloning, expression, sequence analyses of dust mite allergen Der f 6 and its IgE-binding reactivity with mite allergic asthma patients in southeast China. Mol Biol Rep 2012 Feb;39(2):961-8.

[7] An S, Chen L, Wei JF, Yang X, Ma D, Xu X, et al. Purification and characterization of two new allergens from the venom of Vespa magnifica. PLoS One 2011;7(2):e31920.

[8] An S, Ma D, Wei JF, Yang X, Yang HW, Yang H, et al. A novel allergen Tab y 1 with inhibitory activity of platelet aggregation from salivary glands of horseflies. Allergy 2011 Nov;66(11):1420-7.

[9] Caraballo L, Avjioglu A, Marrugo J, Puerta L, Marsh D. Cloning and expression of complementary DNA coding for an allergen with common antibody-binding specificities with three allergens of the house dust mite Blomia tropicalis. J Allergy Clin Immunol 1996 Sep;98(3):573-9.

[10] Caraballo L, Puerta L, Jimenez S, Martinez B, Mercado D, Avjiouglu A, et al. Cloning and IgE binding of a recombinant allergen from the mite Blomia tropicalis, homologous with fatty acid-binding proteins. Int Arch Allergy Immunol 1997 Apr;112(4): 341-7.

[11] Acevedo N, Sanchez J, Erler A, Mercado D, Briza P, Kennedy M, et al. IgE cross-reactivity between Ascaris and domestic mite allergens: the role of tropomyosin and the nematode polyprotein ABA-1. Allergy 2009 Nov;64(11):1635-43.

[12] An S, Chen L, Wei JF, Yang X, Ma D, Xu X, et al. Purification and characterization of two new allergens from the venom of Vespa magnifica. PLoS One 2012;7(2):e31920.

[13] Ayuso R, Grishina D, Bardina L, Carrillo T, Blanco C, Dolores Ibáñez M, et al. Myosin light chain is a novel shrimp allergen, Lit v 3. J Allergy Clin Immunol 2008;122:795-802.

[14] Garcia-Orozco KD, Aispuro-Hernandez E, Yepiz-Plascencia G, Calderon-de-la-Barca AM, Sotelo-Mundo RR. Molecular characterization of arginine kinase, an allergen from the shrimp Litopenaeus vannamei. Int Arch Allergy Immunol 2007;144(1):23-8.

[15] Angus A, Ong S, Chew FT. Sequence Tag catalog of Dust mite-expressed genomes. Am J Pharmacogenomics 2004;4:357-69.

[16] Yi FC, Cheong N, Shek PC, Wang DY, Chua KY, Lee BW. Identification of shared and unique immunoglobulin E epitopes of the highly conserved tropomyosins in Blomia tropicalis and Dermatophagoides pteronyssinus. Clin Exp Allergy 2002 Aug; 32(8):1203-10.

[17] Mora C, Flores I, Montealegre F, Diaz A. Cloning and expression of Blo t 1, a novel allergen from de dust mite Blomia tropicalis, homologous to the cysteine proteases. Clin Exp Allergye 2003;33:28-34.

[18] Puerta L. Obtención y caracterización de un novel alergeno mediante la tecnologia del ADN recombinante Rev Acad Colomb Cienc 2001;25(94):77-87.

[19] Villaverde A, Carrio MM. Protein aggregation in recombinant bacteria: biological role of inclusion bodies. Biotechnol Lett 2003 Sep;25(17):1385-95.

[20] Cassland P, Larsson S, Nilvebrant NO, Jonsson LJ. Heterologous expression of barley and wheat oxalate oxidase in an E. coli trxB gor double mutant. J Biotechnol 2004 Apr 8;109(1-2):53-62.

[21] Lehmann K, Hoffmann S, Neudecker P, Suhr M, Becker WM, Rosch P. High-yield expression in Escherichia coli, purification, and characterization of properly folded major peanut allergen Ara h 2. Protein Expr Purif 2003 Oct;31(2):250-9.

[22] Ponniah K, Loo TS, Edwards PJ, Pascal SM, Jameson GB, Norris GE. The production of soluble and correctly folded recombinant bovine beta-lactoglobulin variants A and B in Escherichia coli for NMR studies. Protein Expr Purif 2010 Apr;70(2):283-9.

[23] Gadermaier G, Harrer A, Girbl T, Palazzo P, Himly M, Vogel L, et al. Isoform identification and characterization of Art v 3, the lipid-transfer protein of mugwort pollen. Mol Immunol 2009 Jun;46(10):1919-24.

[24] Jia D, Li J, Liu L, Zhang D, Yang Y, Du G, et al. High-level expression, purification, and enzymatic characterization of truncated poly(vinyl alcohol) dehydrogenase in methylotrophic yeast Pichia pastoris. Appl Microbiol Biotechnol 2012 Mar 10.

[25] Vu TT, Quyen DT, Dao TT, Nguyen Sle T. Cloning, High-Level Expression, Purification, and Properties of a Novel Endo-beta-1,4-Mannanase from Bacillus subtilis G1 in Pichia pastoris. J Microbiol Biotechnol 2012 Mar;22(3):331-8.

[26] Yi MH, Jeong KY, Kim CR, Yong TS. IgE-binding reactivity of peptide fragments of Bla g 1.02, a major German cockroach allergen. Asian Pac J Allergy Immunol 2009 Jun-Sep;27(2-3):121-9.

[27] Salamanca G, Rodriguez R, Quiralte J, Moreno C, Pascual CY, Barber D, et al. Pectin methylesterases of pollen tissue, a major allergen in olive tree. FEBS J 2010 Jul; 277(13):2729-39.

[28] Goh L-T, Kuo I-C, Luo S, Chua K.Y, White M. Production and purification of recombinant Blomia tropicalis group 5 allergen from Pichia pastoris culture Biotechnology Letters 2001;23(9):661-5.

[29] Labrada M, Uyema K, Sewer M, Labrada A, Gonzalez M, Caraballo L, et al. Monoclonal antibodies against Blo t 13, a recombinant allergen from Blomia tropicalis. Int Arch Allergy Immunol 2002 Nov;129(3):212-8.

[30] Baranyi U, Gattringer M, Boehm A, Marth K, Focke-Tejkl M, Bohle B, et al. Expression of a Major Plant Allergen as Membrane-Anchored and Secreted Protein in Human Cells with Preserved T Cell and B Cell Epitopes. Int Arch Allergy Immunol 2011;156:259-66.

[31] Wagner B, Fuchs H, Adhami F, Ma Y, Scheiner O, Breiteneder H. Plant virus expression systems for transient production of recombinant allergens in Nicotiana benthamiana. Methods 2004;32:227-34.

[32] Hsu CH, Lin SS, Liu FL, Su WC, Yeh SD. Oral administration of a mite allergen expressed by zucchini yellow mosaic virus in cucurbit species downregulates allergen-induced airway inflammation and IgE synthesis. J Allergy Clin Immunol 2004;113:1079-85.

[33] Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TK, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. Nature 2010 Apr 29;464(7293):1367-70.

[34] Barlow JL, Bellosi A, Hardman CS, Drynan LF, Wong SH, Cruickshank JP, et al. Innate IL-13-producing nuocytes arise during allergic lung inflammation and contribute to airways hyperreactivity. J Allergy Clin Immunol 2012 Jan;129(1):191-8 e1-4.

[35] Sokol CL, Barton GM, Farr AG, Medzhitov R. A mechanism for the initiation of allergen-induced T helper type 2 responses. Nat Immunol 2008 Mar;9(3):310-8.

[36] Saenz SA, Siracusa MC, Perrigoue JG, Spencer SP, Urban JF, Jr., Tocker JE, et al. IL25 elicits a multipotent progenitor cell population that promotes T(H)2 cytokine responses. Nature 2010 Apr 29;464(7293):1362-6.

[37] Veldhoen M, Uyttenhove C, van Snick J, Helmby H, Westendorf A, Buer J, et al. Transforming growth factor-beta 'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. Nat Immunol 2008 Dec;9(12): 1341-6.

[38] Zhao Y, Yang J, Gao YD, Guo W. Th17 immunity in patients with allergic asthma. Int Arch Allergy Immunol 2010;151(4):297-307.

[39] Durham SR, Walker SM, Varga EM, Jacobson MR, O'Brien F, Noble W, et al. Long-term clinical efficacy of grass-pollen immunotherapy. N Engl J Med 1999 Aug 12;341(7):468-75.

[40] Frew AJ, Powell RJ, Corrigan CJ, SR D. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006;117:319–25.

[41] Akdis M, Blaser K, Akdis CA. T regulatory cells in allergy: novel concepts in the pathogenesis, prevention, and treatment of allergic diseases. J Allergy Clin Immunol 2005 Nov;116(5):961-8; quiz 9.

[42] Jutel M, Akdis M, Budak F, Aebischer-Casaulta C, Wrzyszcz M, Blaser K, et al. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. Eur J Immunol 2003 May;33(5):1205-14.

[43] Jutel M, Jaeger L, Suck R, Meyer H, Fiebig H, Cromwell O. Allergen-specific immunotherapy with recombinant grass pollen allergens. J Allergy Clin Immunol 2005 Sep;116(3):608-13.

[44] Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell 2008 May 30;133(5):775-87.

[45] Izcue A, Hue S, Buonocore S, Arancibia-Carcamo CV, Ahern PP, Iwakura Y, et al. Interleukin-23 restrains regulatory T cell activity to drive T cell-dependent colitis. Immunity 2008 Apr;28(4):559-70.

[46] Akdis CA, Kussebi F, Pulendran B, Akdis M, Lauener RP, Schmidt-Weber CB, et al. Inhibition of T helper 2-type responses, IgE production and eosinophilia by synthetic lipopeptides. Eur J Immunol 2003 Oct;33(10):2717-26.

[47] Reisinger J, Horak F, Pauli G, van Hage M, Cromwell O, Konig F, et al. Allergen-specific nasal IgG antibodies induced by vaccination with genetically modified allergens are associated with reduced nasal allergen sensitivity. J Allergy Clin Immunol 2005 Aug;116(2):347-54.

[48] Strait RT, Morris SC, Finkelman FD. IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and Fc gamma RIIb cross-linking. J Clin Invest 2006 Mar;116(3):833-41.

[49] Finkelman MA, Lempitski SJ, Slater JE. beta-Glucans in standardized allergen extracts. J Endotoxin Res 2006;12(4):241-5.

[50] Focke M, Marth K, Valenta R. Molecular composition and biological activity of commercial birch pollen allergen extracts. Eur J Clin Invest 2009 May;39(5):429-36.

[51] Focke M, Marth K, Flicker S, Valenta R. Heterogeneity of commercial timothy grass pollen extracts. Clin Exp Allergy 2008 Aug;38(8):1400-8.

[52] Trivedi B, Valerio C, Slater JE. Endotoxin content of standardized allergen vaccines. J Allergy Clin Immunol 2003 Apr;111(4):777-83.

[53] Traidl-Hoffmann C, Mariani V, Hochrein H, Karg K, Wagner H, Ring J, et al. Pollen-associated phytoprostanes inhibit dendritic cell interleukin-12 production and augment T helper type 2 cell polarization. J Exp Med 2005 Feb 21;201(4):627-36.

[54] Moverare R, Elfman L, Vesterinen E, Metso T, Haahtela T. Development of new IgE specificities to allergenic components in birch pollen extract during specific immunotherapy studied with immunoblotting and Pharmacia CAP System. Allergy 2002 May;57(5):423-30.

[55] Cromwell O, Hafner D, Nandy A. Recombinant allergens for specific immunotherapy. J Allergy Clin Immunol 2011 Apr;127(4):865-72.

[56] Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Gronlund H. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). Clin Exp Allergy 1999 Jul;29(7):896-904.

[57] Ballmer-Weber BK, Scheurer S, Fritsche P, Enrique E, Cistero-Bahima A, Haase T, et al. Component-resolved diagnosis with recombinant allergens in patients with cherry allergy. J Allergy Clin Immunol 2002 Jul;110(1):167-73.

[58] Astier C, Morisset M, Roitel O, Codreanu F, Jacquenet S, Franck P, et al. Predictive value of skin prick tests using recombinant allergens for diagnosis of peanut allergy. J Allergy Clin Immunol 2006 Jul;118(1):250-6.

[59] Bauermeister K, Ballmer-Weber BK, Bublin M, Fritsche P, Hanschmann KM, Hoffmann-Sommergruber K, et al. Assessment of component-resolved in vitro diagnosis of celeriac allergy. J Allergy Clin Immunol 2009 Dec;124(6):1273-81 e2.

[60] Akkerdaas JH, Wensing M, Knulst AC, Krebitz M, Breiteneder H, de Vries S, et al. How accurate and safe is the diagnosis of hazelnut allergy by means of commercial skin prick test reagents? Int Arch Allergy Immunol 2003 Oct;132(2):132-40.

[61] Dalmau Duch G, Gazquez Garcia V, Gaig Jane P, Galan Nieto A, Monsalve Clemente RI. Importance of controlled sting challenge and component-resolved diagnosis in the success of venom immunotherapy. J Investig Allergol Clin Immunol 2012;22(2):135-6.

[62] Monsalve RI, Vega A, Marques L, Miranda A, Fernandez J, Soriano V, et al. Component-resolved diagnosis of vespid venom-allergic individuals: phospholipases and antigen 5s are necessary to identify Vespula or Polistes sensitization. Allergy 2012 Apr;67(4):528-36.

[63] Tripodi S, Frediani T, Lucarelli S, Macri F, Pingitore G, Di Rienzo Businco A, et al. Molecular profiles of IgE to Phleum pratense in children with grass pollen allergy: implications for specific immunotherapy. J Allergy Clin Immunol 2012 Mar;129(3):834-9 e8.

[64] Metz-Favre C, Linhart B, Focke-Tejkl M, Purohit A, de Blay F, Valenta R, et al. Skin test diagnosis of grass pollen allergy with a recombinant hybrid molecule. J Allergy Clin Immunol 2007 Aug;120(2):315-21.

[65] Hueber W, Utz PJ, Robinson WH. Autoantibodies in early arthritis: advances in diagnosis and prognostication. Clin Exp Rheumatol 2003 Sep-Oct;21(5 Suppl 31):S59-64.

[66] Gadermaier G, Wopfner N, Wallner M, Egger M, Didierlaurent A, Regl G, et al. Array-based profiling of ragweed and mugwort pollen allergens. Allergy 2008 Nov; 63(11):1543-9.

[67] Ott H, Folster-Holst R, Merk HF, Baron JM. Allergen microarrays: a novel tool for high-resolution IgE profiling in adults with atopic dermatitis. Eur J Dermatol 2010 Jan-Feb;20(1):54-61.

[68] Jahn-Schmid B, Harwanegg C, Hiller R, Bohle B, Ebner C, Scheiner O, et al. Allergen microarray: comparison of microarray using recombinant allergens with conventional diagnostic methods to detect allergen-specific serum immunoglobulin E Clin Exp Allergy 2003;33.

[69] Ebo DG, Hagendorens MM, De Knop KJ, Verweij MM, Bridts CH, De Clerck LS, et al. Component-resolved diagnosis from latex allergy by microarray. Clin Exp Allergy 2010 Feb;40(2):348-58.

[70] Perry TT, Matsui EC, Conover-Walker MK, Wood RA. Risk of oral food challenges. J Allergy Clin Immunol 2004 Nov;114(5):1164-8.

[71] Lin J, Bruni FM, Fu Z, Maloney J, Bardina L, Boner AL, et al. A bioinformatics approach to identify patients with symptomatic peanut allergy using peptide microarray immunoassay. J Allergy Clin Immunol 2012 May;129(5):1321-8 e5.

[72] Jimenez S, Puerta L, Mendoza D, K.W.Chua, Mercado D, Caraballo L. IgE Antibody Responses to Recobinant Allergens of Blomia tropicalis and Dermatophagoides pteronyssinus in a Tropical Enviroment. Allergy Clin Immunol Int - J World Allergy Org 2007;19:233-8.

[73] Pauli G, Oster JP, Deviller P, Heiss S, Bessot JC, Susani M, et al. Skin testing with recombinant allergens rBet v 1 and birch profilin, rBet v 2: diagnostic value for birch pollen and associated allergies. J Allergy Clin Immunol 1996 May;97(5):1100-9.

[74] Godnic-Cvar J, Susani M, Breiteneder H, Berger A, Havelec L, Waldhor T, et al. Recombinant Bet v 1, the major birch pollen allergen, induces hypersensitivity reactions equal to those induced by natural Bet v 1 in the airways of patients allergic to tree pollen. J Allergy Clin Immunol 1997 Mar;99(3):354-9.

[75] Pauli G, Larsen TH, Rak S, Horak F, Pastorello E, Valenta R, et al. Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis. J Allergy Clin Immunol 2008 Nov;122(5):951-60.

[76] Focke M, Swoboda I, Marth K, Valenta R. Developments in allergen-specific immunotherapy: from allergen extracts to allergy vaccines bypassing allergen-specific immunoglobulin E and T cell reactivity. Clin Exp Allergy 2010 Mar;40(3):385-97.

[77] Ferreira F, Hirtenlehner K, Jilek A, Godnik-Cvar J, Breiteneder H, Grimm R, et al. Dissection of immunoglobulin E and T lymphocyte reactivity of isoforms of the major birch pollen allergen Bet v 1: potential use of hypoallergenic isoforms for immunotherapy. J Exp Med 1996 Feb 1;183(2):599-609.

[78] Arquint O, Helbling A, Crameri R, Ferreira F, Breitenbach M, Pichler WJ. Reduced in vivo allergenicity of Bet v 1d isoform, a natural component of birch pollen. J Allergy Clin Immunol 1999 Dec;104(6):1239-43.

[79] Wagner S, Radauer C, Bublin M, Hoffmann-Sommergruber K, Kopp T, Greuseneger EK, et al. Naturally ocurring hypoallergenic Bet v 1 isoforms fail to induce IgE responses in individuals with birch pollen allergy. J Allergy Clin Immunol 2008;121:246-52.

[80] Chapman MD, Smith AM, Vailes LD, Arruda LK, Dhanaraj V, Pomes A. Recombinant allergens for diagnosis and therapy of allergic disease. J Allergy Clin Immunol 2000 Sep;106(3):409-18.

[81] Sharrow SD, Edmonds KA, Goodman MA, Novotny MV, Stone MJ. Thermodynamic consequences of disrupting a water-mediated hydrogen bond network in a protein:pheromone complex. Protein Sci 2005 Jan;14(1):249-56.

[82] Ferrari E, Breda D, Longhi R, Vangelista L, Nakaie CR, Elviri L, et al. In search of a vaccine for mouse allergy: significant reduction of Mus m 1 allergenicity by structure-guided single-point mutations. Int Arch Allergy Immunol 2012;157(3):226-37.

[83] Gadermaier G, Jahn-Schmid B, Vogel L, Egger M, Himly M, Briza P, et al. Targeting the cysteine-stabilized fold of Art v 1 for immunotherapy of Artemisia pollen allergy. Mol Immunol 2010 Mar;47(6):1292-8.

[84] Spangfort MD, Mirza O, Ipsen H, Van Neerven RJ, Gajhede M, Larsen JN. Dominating IgE-binding epitope of Bet v 1, the major allergen of birch pollen, characterized by X-ray crystallography and site-directed mutagenesis. J Immunol 2003 Sep 15;171(6):3084-90.

[85] King TP, Jim SY, Monsalve RI, Kagey-Sobotka A, Lichtenstein LM, Spangfort MD. Recombinant allergens with reduced allergenicity but retaining immunogenicity of the natural allergens: hybrids of yellow jacket and paper wasp venom allergen antigen 5s. J Immunol 2001 May 15;166(10):6057-65.

[86] Gonzalez-Rioja R, Ibarrola I, Arilla MC, Ferrer A, Mir A, Andreu C, et al. Genetically engineered hybrid proteins from Parietaria judaica pollen for allergen-specific immunotherapy. J Allergy Clin Immunol 2007 Sep;120(3):602-9.

[87] Linhart B, Hartl A, Jahn-Schmid B, Verdino P, Keller W, Krauth MT, et al. A hybrid molecule resembling the epitope spectrum of grass pollen for allergy vaccination. J Allergy Clin Immunol 2005 May;115(5):1010-6.

[88] Asturias JA, Ibarrola I, Arilla MC, Vidal C, Ferrer A, Gamboa PM, et al. Engineering of major house dust mite allergens Der p 1 and Der p 2 for allergen-specific immunotherapy. Clin Exp Allergy 2009 Jul;39(7):1088-98.

[89] Nouri HR, Varasteh A, Vahedi F, Chamani J, Afsharzadeh D, Sankian M. Constructing a hybrid molecule with low capacity of IgE binding from Chenopodium album pollen allergens. Immunol Lett 2012 May 30;144(1-2):67-77.

[90] Ball T, Linhart B, Sonneck K, Blatt K, Herrmann H, Valent P, et al. Reducing allergenicity by altering allergen fold: a mosaic protein of Phl p 1 for allergy vaccination. Allergy 2009 Apr;64(4):569-80.

[91] Mothes-Luksch N, Stumvoll S, Linhart B, Focke M, Krauth MT, Hauswirth A, et al. Disruption of allergenic activity of the major grass pollen allergen Phl p 2 by reassembly as a mosaic protein. J Immunol 2008 Oct 1;181(7):4864-73.

[92] Campana R, Vrtala S, Maderegger B, Jertschin P, Stegfellner G, Swoboda I, et al. Hypoallergenic derivatives of the major birch pollen allergen Bet v 1 obtained by rational sequence reassembly. J Allergy Clin Immunol 2010 Nov;126(5):1024-31, 31 e1-8.

[93] Chen KW, Fuchs G, Sonneck K, Gieras A, Swoboda I, Douladiris N, et al. Reduction of the in vivo allergenicity of Der p 2, the major house-dust mite allergen, by genetic engineering. Mol Immunol 2008 May;45(9):2486-98.

[94] Vrtala S, Akdis CA, Budak F, Akdis M, Blaser K, Kraft D, et al. T cell epitope-containing hypoallergenic recombinant fragments of the major birch pollen allergen, Bet v 1, induce blocking antibodies. J Immunol 2000 Dec 1;165(11):6653-9.

[95] Pauli G, Purohit A, Oster JP, De Blay F, Vrtala S, Niederberger V, et al. Comparison of genetically engineered hypoallergenic rBet v 1 derivatives with rBet v 1 wild-type by skin prick and intradermal testing: results obtained in a French population. Clin Exp Allergy 2000 Aug;30(8):1076-84.

[96] Focke M, Linhart B, Hartl A, Wiedermann U, Sperr WR, Valent P, et al. Non-anaphylactic surface-exposed peptides of the major birch pollen allergen, Bet v 1, for preventive vaccination. Clin Exp Allergy 2004 Oct;34(10):1525-33.

[97] Vrtala S, Hirtenlehner K, Susani M, Akdis M, Kussebi F, Akdis CA, et al. Genetic engineering of a hypoallergenic trimer of the major birch pollen allergen Bet v 1. FASEB J 2001 Sep;15(11):2045-7.

[98] Johansson J, Hellman L. Modifications increasing the efficacy of recombinant vaccines; marked increase in antibody titers with moderately repetitive variants of a therapeutic allergy vaccine. Vaccine 2007 Feb 19;25(9):1676-82.

[99] Vrtala S, Fohr M, Campana R, Baumgartner C, Valent P, Valenta R. Genetic engineering of trimers of hypoallergenic fragments of the major birch pollen allergen, Bet v 1, for allergy vaccination. Vaccine 2011 Mar 3;29(11):2140-8.

[100] Calderon MA, Larenas D, Kleine-Tebbe J, Jacobsen L, Passalacqua G, Eng PA, et al. European Academy of Allergy and Clinical Immunology task force report on 'dose-response relationship in allergen-specific immunotherapy'. Allergy 2011 Oct;66(10): 1345-59.

[101] Blaser K. Allergen dose dependent cytokine production regulates specific IgE and IgG antibody production. Adv Exp Med Biol 1996;409:295-303.

[102] Fittipaldi A, Giacca M. Transcellular protein transduction using the Tat protein of HIV-1. Adv Drug Deliv Rev 2005 Feb 28;57(4):597-608.

[103] Herce HD, Garcia AE. Molecular dynamics simulations suggest a mechanism for translocation of the HIV-1 TAT peptide across lipid membranes. Proc Natl Acad Sci U S A 2007 Dec 26;104(52):20805-10.

[104] Rhyner C, Kundig T, Akdis CA, Crameri R. Targeting the MHC II presentation pathway in allergy vaccine development. Biochem Soc Trans 2007 Aug;35(Pt 4):833-4.

[105] Crameri R, Fluckiger S, Daigle I, Kundig T, Rhyner C. Design, engineering and in vitro evaluation of MHC class-II targeting allergy vaccines. Allergy 2007 Feb;62(2): 197-206.

[106] Martinez-Gomez JM, Johansen P, Rose H, Steiner M, Senti G, Rhyner C, et al. Targeting the MHC class II pathway of antigen presentation enhances immunogenicity and safety of allergen immunotherapy. Allergy 2009 Jan;64(1):172-8.

[107] Lutz MB, Schuler G. Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity? Trends Immunol 2002 Sep;23(9):445-9.

[108] Rutella S, Danese S, Leone G. Tolerogenic dendritic cells: cytokine modulation comes of age. Blood 2006 Sep 1;108(5):1435-40.

[109] Novak N, Valenta R, Bohle B, Laffer S, Haberstok J, Kraft S, et al. FcepsilonRI engagement of Langerhans cell-like dendritic cells and inflammatory dendritic epidermal cell-like dendritic cells induces chemotactic signals and different T-cell phenotypes in vitro. J Allergy Clin Immunol 2004 May;113(5):949-57.

[110] Zhu D, Kepley CL, Zhang K, Terada T, Yamada T, Saxon A. A chimeric human-cat fusion protein blocks cat-induced allergy. Nat Med 2005 Apr;11(4):446-9.

[111] Terada T, Zhang K, Belperio J, Londhe V, Saxon A. A chimeric human-cat Fcgamma-Fel d1 fusion protein inhibits systemic, pulmonary, and cutaneous allergic reactivity to intratracheal challenge in mice sensitized to Fel d1, the major cat allergen. Clin Immunol 2006 Jul;120(1):45-56.

[112] Lin LH, Zheng P, Yuen JW, Wang J, Zhou J, Kong CQ, et al. Prevention and treatment of allergic inflammation by an Fcgamma-Der f2 fusion protein in a murine model of dust mite-induced asthma. Immunol Res 2012 Jun;52(3):276-83.

[113] Vailes LD, Sun AW, Ichikawa K, Wu Z, Sulahian TH, Chapman MD, et al. High-level expression of immunoreactive recombinant cat allergen (Fel d 1): Targeting to antigen-presenting cells. J Allergy Clin Immunol 2002 Nov;110(5):757-62.

[114] Hulse KE, Reefer AJ, Engelhard VH, Satinover SM, Patrie JT, Chapman MD, et al. Targeting Fel d 1 to FcgammaRI induces a novel variation of the T(H)2 response in subjects with cat allergy. J Allergy Clin Immunol 2008 Mar;121(3):756-62 e4.

[115] Hulse KE, Reefer AJ, Engelhard VH, Patrie JT, Ziegler SF, Chapman MD, et al. Targeting allergen to FcgammaRI reveals a novel T(H)2 regulatory pathway linked to thymic stromal lymphopoietin receptor. J Allergy Clin Immunol 2010 Jan;125(1): 247-56 e1-8.

[116] Muller U, Golden DB, Lockey RF, Shin B. Immunotherapy for hymenoptera venom hypersensitivity. Clin Allergy Immunol 2008;21:377-92.

[117] Bilo BM, Bonifazi F. Epidemiology of insect-venom anaphylaxis. Curr Opin Allergy Clin Immunol 2008 Aug;8(4):330-7.

[118] Graft DF. Insect sting allergy. Med Clin North Am 2006 Jan;90(1):211-32.

[119] Brasch J, Maidusch T. Immunotherapy with wasp venom is accompanied by wide-ranging immune responses that need further exploration. Acta Derm Venereol 2009;89(5):466-9.

[120] Mosbech H, Muller U. Side-effects of insect venom immunotherapy: results from an EAACI multicenter study. European Academy of Allergology and Clinical Immunology. Allergy 2000 Nov;55(11):1005-10.

[121] Kammerer R, Chvatchko Y, Kettner A, Dufour N, Corradin G, Spertini F. Modulation of T-cell response to phospholipase A2 and phospholipase A2-derived peptides by conventional bee venom immunotherapy. J Allergy Clin Immunol 1997 Jul;100(1): 96-103.

[122] Muller U, Akdis CA, Fricker M, Akdis M, Blesken T, Bettens F, et al. Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. J Allergy Clin Immunol 1998 Jun;101(6 Pt 1):747-54.

[123] Fellrath JM, Kettner A, Dufour N, Frigerio C, Schneeberger D, Leimgruber A, et al. Allergen-specific T-cell tolerance induction with allergen-derived long synthetic peptides: results of a phase I trial. J Allergy Clin Immunol 2003 Apr;111(4):854-61.

[124] Winkler B, Bolwig C, Seppala U, Spangfort MD, Ebner C, Wiedermann U. Allergen-specific immunosuppression by mucosal treatment with recombinant Ves v 5, a ma-

jor allergen of Vespula vulgaris venom, in a murine model of wasp venom allergy. Immunology 2003 Nov;110(3):376-85.

[125] Kussebi F, Karamloo F, Rhyner C, Schmid-Grendelmeier P, Salagianni M, Mannhart C, et al. A major allergen gene-fusion protein for potential usage in allergen-specific immunotherapy. J Allergy Clin Immunol 2005 Feb;115(2):323-9.

[126] Cascone O, Amaral V, Ferrara P, Vita N, Guillemot JC, Diaz LE. Purification and characterization of two forms of antigen 5 from polybia scutellaris venom. Toxicon 1995 May;33(5):659-65.

[127] Pirpignani ML, Rivera E, Hellman U, Biscoglio de Jimenez Bonino M. Structural and immunological aspects of Polybia scutellaris Antigen 5. Arch Biochem Biophys 2002 Nov 15;407(2):224-30.

[128] Vinzon SE, Marino-Buslje C, Rivera E, Biscoglio de Jimenez Bonino M. A Naturally Occurring Hypoallergenic Variant of Vespid Antigen 5 from Polybia scutellaris Venom as a Candidate for Allergen-Specific Immunotherapy. PLoS One 2012;7(7):e41351.

[129] Haselden BM, Kay AB, Larche M. Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. J Exp Med 189 1999;189:1885-94.

[130] Norman PS, Ohman JL, Jr., Long AA, Creticos PS, Gefter MA, Shaked Z, et al. Treatment of cat allergy with T-cell reactive peptides. Am J Respir Crit Care Med 1996 Dec;154(6 Pt 1):1623-8.

[131] Simons FE, Imada M, Li Y, Watson WT, HayGlass KT. Fel d 1 peptides: effect on skin tests and cytokine synthesis in cat-allergic human subjects. Int Immunol 1996 Dec; 8(12):1937-45.

[132] Maguire P, Nicodemus C, Robinson D, Aaronson D, Umetsu DT. The safety and efficacy of ALLERVAX CAT in cat allergic patients. Clin Immunol 1999 Dec;93(3):222-31.

[133] Haselden BM, Larche M, Meng Q, Shirley K, Dworski R, Kaplan AP, et al. Late asthmatic reactions provoked by intradermal injection of T-cell peptide epitopes are not associated with bronchial mucosal infiltration of eosinophils or T(H)2-type cells or with elevated concentrations of histamine or eicosanoids in bronchoalveolar fluid. J Allergy Clin Immunol 2001 Sep;108(3):394-401.

[134] Oldfield WL, Kay AB, Larche M. Allergen-derived T cell peptide-induced late asthmatic reactions precede the induction of antigen-specific hyporesponsiveness in atopic allergic asthmatic subjects. J Immunol 2001 Aug 1;167(3):1734-9.

[135] Ali FR, Oldfield WL, Higashi N, Larche M, Kay AB. Late asthmatic reactions induced by inhalation of allergen-derived T cell peptides. Am J Respir Crit Care Med 2004 Jan 1;169(1):20-6.

[136] Niederberger V, Horak F, Vrtala S, Spitzauer S, Krauth MT, Valent P, et al. Vaccination with genetically engineered allergens prevents progression of allergic disease. Proc Natl Acad Sci U S A 2004;101(suppl 2):14677-82.

[137] Winther L, Poulsen LK, Robin B, Mélac M, Malling H. Safety and Tolerability of Recombinant Bet v 1 (rBet v 1) tablets in sublingual immunotherapy (SLIT). J Allergy Clin Immunol 2009;123(suppl):S215.

[138] Senti G, Crameri R, Kuster D, Johansen P, Martinez-Gomez JM, Graf N, et al. Intralymphatic immunotherapy for cat allergy induces tolerance after only 3 injections. J Allergy Clin Immunol 2012 May;129(5):1290-6.

[139] Tulic MK, Fiset PO, Christodoulopoulos P, Vaillancourt P, Desrosiers M, Lavigne F, et al. Amb a 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy decreases the nasal inflammatory response. J Allergy Clin Immunol 2004 Feb;113(2): 235-41.

[140] Asai K, Foley SC, Sumi Y, Yamauchi Y, Takeda N, Desrosiers M, et al. Amb a 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy increases CD4+CD25+ T cells in the nasal mucosa of subjects with allergic rhinitis. Allergol Int 2008 Dec;57(4):377-81.

[141] Linneberg A, Gislum M, Johansen N, Husemoen LL, Jorgensen T. Temporal trends of aeroallergen sensitization over twenty-five years. Clin Exp Allergy 2007 Aug;37(8): 1137-42.

[142] Boulet LP, Turcotte H, Laprise C, Lavertu C, Bedard PM, Lavoie A, et al. Comparative degree and type of sensitization to common indoor and outdoor allergens in subjects with allergic rhinitis and/or asthma. Clin Exp Allergy 1997 Jan;27(1):52-9.

[143] Tsai JJ, Wu HH, Shen HD, Hsu EL, Wang SR. Sensitization to Blomia tropicalis among asthmatic patients in Taiwan. Int Arch Allergy Immunol 1998 Feb;115(2): 144-9.

[144] Fernandez-Caldas E, Baena-Cagnani CE, Lopez M, Patino C, Neffen HE, Sanchez-Medina M, et al. Cutaneous sensitivity to six mite species in asthmatic patients from five Latin American countries. J Investig Allergol Clin Immunol 1993 Sep-Oct;3(5): 245-9.

[145] Puerta L, Fernandez-Caldas E, Lockey RF, Caraballo LR. Mite allergy in the tropics: sensitization to six domestic mite species in Cartagena, Colombia. J Investig Allergol Clin Immunol 1993 Jul-Aug;3(4):198-204.

[146] Shen HD, Chua KY, Lin KL, Shich KH, Thomas WR. Molecular cloning of house dust mite allergens with common antibody binding-specificities with multiple components in mite extracts. Int Arch Allergy Immunol 1993;23:934-40.

[147] Puerta L, Lagares A, Mercado D, Fernandez-Caldas E, Caraballo L. Allergenic composition of the mite Suidasia medanensis and cross-reactivity with Blomia tropicalis. Allergy 2005 Jan;60(1):41-7.

[148] Weghofer M, Thomas WR, Kronqvist M, Mari A, Purohit A, Pauli G, et al. Variability of IgE reactivity profiles among European mite allergic patients. Eur J Clin Invest 2008 Dec;38(12):959-65.

[149] Lynch NR, Thomas WR, Garcia NM, Di Prisco MC, Puccio FA, L'Opez R I, et al. Biological activity of recombinant Der p 2, Der p 5 and Der p 7 allergens of the house-dust mite Dermatophagoides pteronyssinus. Int Arch Allergy Immunol 1997 Sep; 114(1):59-67.

[150] Thomas WR, Hales BJ, Smith WA. Structural biology of allergens. Curr Allergy Asthma Rep 2005 Sep;5(5):388-93.

[151] Pittner G, Vrtala S, Thomas WR, Weghofer M, Kundi M, Horak F, et al. Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. Clin Exp Allergy 2004 Apr;34(4):597-603.

[152] Zakzuk J, Jimenez S, Cheong N, Puerta L, Lee BW, Chua KY, et al. Immunlogical characterization of Blo t 12 isoallergen: identification of immunoglobulin E epitopes. Clin Exp Allergy 2009;39:608-16.

[153] Puerta L, Martinez D, Munera M, Cantillo J, Caraballo L. Recombinant protein assembling epitopes from different allergens of Dermatophagoides pteronyssinus. Presented at: European Academy of Allergy and Clinical Immunology Congress. June 16-20, 2012. Geneve, Switzerland.

IntechOpen

IntechOpen

IntechOpen