Human Vaccines & Immunotherapeutics 8:10, 1534-1543; October 2012; © 2012 Landes Bioscience

Recombinant allergens
The present and the future

Marek Jutel,* Katarzyna Solarewicz-Madejek and Sylwia Smolinska

Department of Clinical Immunology; Wroclaw Medical University; Wroclaw, Poland

Keywords: allergy, allergen-specific immunotherapy, recombinant allergens, hypoallergens, vaccines

Abbreviations: SIT, specific immunotherapy; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; DBPC, double-blind placebo-controlled

Allergen specific immunotherapy (SIT) is the only known causative treatment of allergic diseases. Recombinant allergen-based vaccination strategies arose from a strong need to both to improve safety and enhance efficacy of SIT. In addition, new vaccines can be effective in allergies including food allergy or atopic dermatitis, which poorly respond to the current treatment with allergen extracts. A number of successful clinical studies with both wild-type and hypoallergenic derivatives of recombinant allergens vaccines have been reported for the last decade. They showed high efficacy and safety profile as well as very strong modulation of T and B cell responses to specific allergens.

Introduction

Allergen Specific Immunotherapy (SIT) is the only attempt to cure allergic diseases by using allergen -specific immunological mechanisms. The treatment is currently under intense development in several aspects including: allergen tyoe and content, adjuvantroute of administration, protocols of desensitization etc. Allergen-specific immunotherapy is a very effective in the treatment of the type I allergic diseases by both reducing the symptoms of allergic rhinitis and/ or asthma, and the use of symptom relieving medication as well as improving the quality of life. Importantly SIT shows long-lasting benefits, even after cessation of the treatment. It has been also shown, that SIT can reduce new allergen sensitization risk[1] as well as prevent development of bronchial asthma in allergic individuals.[2] Several mechanisms have been proposed to explain the beneficial effects of immunotherapy The long-term allergen tolerance is achieved by modulation of allergen-specific memory T- and B-cell responses as well suppression of effector mechanisms. Cellular and molecular mechanisms have been demonstrated during an effective SIT, which include: increase in allergen-specific suppressive capacities of both inducible subsets of $CD4^+$ $CD25^+$ forkhead box $P3^+$ ($FoxP3^+$) T-regulatory and IL-10-secreting type 1 T-regulatory cellse in the peripheral blood, suppression of eosinophils, mast cells and basophiles, as well as the antibody (Ab) isotype change from immunoglobulin (Ig) IgE to IgG_4.[3,4]

The pioneer clinical trials with the allergen SIT were undertaken by Noon in 1911[5] and continued by Freeman in Europe in the grass pollen seasonal allergic rhinitis ("hay fever"). The allergens used in the therapy were plainly water-extracted from grass pollen mixture. However effective while used in allergy therapy, carried a high risk of serious adverse events in patients during the allergen desensitization.[6] In 1940s, depot- or semi-depot preparations of the allergen extracts were elaborated. The adjuvants (commonly used aluminum hydroxide), which are commonly used in vaccines, allow to reduce the number of injections as well as to increase the immunotherapy effectiveness, and to limit the therapy-related severe adverse events.[7]

The research to improve efficacy and safety of SIT resulted in introduction of allergoids to the allergy specific treatment in the 1970s.[6] The allergoids are prepared by chemical modification of the allergenic proteins with use of either formaldehyde or glutaraldehyde. The aldehydes acts as protein cross-linkers, producing high-molecular-weight allergen polymers.[8,9] Such modified polymeric molecules show the same amount of immunogenicity, but less allergenicity, with lessened unwanted reactions ratio, due to destruction of IgE epitopes in the allergens and are usually administered after absorbtion on adjuvants, like aluminiumhydroxide (Alum).[6]

The next step to reduce the unwanted side effects and improve quality of the vaccines was standardization of the extracts. The standardization process allows for production of well-characterized, non-contaminated extracts of known biologic potency and composition.[10] Currently used allergen extracts are standardized for total allergenic activity and the content of major allergens, but they contain many proteins which are not allergens. One particular allergen source contains usually more than one clinically relevant allergen and constitutes a set of major and minor allergens of different clinical importance.[11]

The composition of allergen extracts is determined to a large extent by the quality of the raw material and the method of extraction and purification.

Different other options to access the goal of effective and safe SIT have been reported. The addition of omalizumab, an anti-IgE recombinant humanized monoclonal Ab (mAb) approved

*Correspondence to: Marek Jutel; Email: marek.jutel@am.wroc.pl
Submitted: 06/28/12; Revised: 08/29/12; Accepted: 09/03/12
http://dx.doi.org/10.4161/hv.22064

for use in patients with moderate-to-severe perennial allergic asthma, to subcutaneous immunotherapy (SCIT) showed benefit in the patients in terms of SIT-related systemic and severe reactions. However the data obtained indicate that omalizumab pretreatment increased SCIT safety, the mechanism is not clear and the studies have been too short-term to finally determine this issue.[12] Toll-like receptor (TLR) agonists designed to respond to a variety of pathogens and to induce T helper 1 (Th1) and regulatory T-cell (Treg) responses, applied either in combination with allergen or alone, might also be useful in designing a novel and effective immunotherapy in the future.[12]

Confirmed clinical relevance of a particular allergen protein in a patient accounts for a possibility of individually tailored allergen-specific immunotherapy. Compared with allergen extracts, the recombinant allergens cocktails can be composed with high quality and precise quantity of relevant allergen as well as with high technical reproducibility of the mixture. The component-resolved immunotherapy preparations may be based on the individually composed mixtures of allergenic epitopes, however more immediate commercial prospect is the application of standard allergen cocktails derived from one source, or in some cases, like for *Fel d 1*, *Art v1* or *Bet v 1* – dependent allergy, containing single relevant allergen.

Other techniques to create hypoallergenic extracts included point mutations on the allergen IgE binding site, reducing the IgE-mediated effects; major allergens fusions, such as bee venom *Api m 1* and *Api m 2*, to delete B-cell epitopes while preserving T-cell epitopes; DNA shuffling to maintain the T-cell epitopes but decrease the allergenicity of the substance;[12] bacterial cell surface (S-layer) protein and the major birch pollen allergen *Bet v 1* fusion (rSbsC-Bet v 1) to enhance the Th1-like immune response.[13]

Another modification of molecules introduced into the allergy research is application of allergenic peptides. The peptides loose the features of IgE-binding epitopes while retain features of the T lymphocyte epitopes. Aimed to target exclusively the T cell receptor (TCR) are supposed to increase the efficacy of the immune tolerance induction and to reduce the IgE-mediated side effect of the immunotherapy.[14] The use of recombinant DNA technology appears to provide a realistic means of achieving improvements in obtaining precisely defined preparations.[15] The genetic modifications result in hypoallergenicity of the allergen derivatives—recombinant allergens and peptides. Natural allergen extracts can thus be substituted by pure recombinant wild-type allergens or single recombinant fusion proteins consisting of several wild-type allergen.[16]

Different routes of the SIT delivery have been also under investigation to improve SIT safety and efficacy, such as oral, sublingual, nasal, bronchial, epicutaneous, intraepithelial and intralymphatic.[12]

Recombinant Allergens Design—Allergen Engineering

Establishing the protein molecular structure as well as the immune function of a certain natural allergen and its epitopes enabled cloning of allergen proteins with use of recombinant DNA technology.[17] The general steps of the DNA cloning are the "reverse transcription" of the protein encrypting mRNA to a cDNA strand (cDNA), with the gene of interest, and its subsequent transfer into a microorganism's genome or plasmid, like bacterium *Escherichia coli* (*E. coli*) or yeast Pichia pastoris. Each bacterium produces 10 to 100 copies of the incorporated DNA. The genetic material could be than analyzed and engineered. The most known techniques that allowed to work on low-abundance mRNA proteins, are bacteriophages cDNA libraries based methods[18] and microsequencing of the proteins.[19]

The first allergen proteins were cloned in 1988: a cDNA coding *Der p 1*, a house dust mite *Dermatophagoides pteronyssinus* major allergen number 1[20,21] and a white-face hornet (*Dolichovespula maculata*) major allergen number 5 (*Dol m 5*).[22] The first recombinant allergens were synthetized with no pretranslational interference in their molecular structure and presented the same or very similar allergenicity as the wild-type proteins, assessed in skin tests, serum IgE-binding assays or basophil degranulation tests, as reported for recombinant house dust mite *Dermatophagoides farinae* allergen 2 (*Der p 2*), birch *Betula verrucosa* allergen 1 and 2 (*Bet v 1, Bet v 2*), timothy grass *Phleum pratense* allergen 2 (*Phl p 2*).[19] However, some of the recombinant proteins vary in similarity to their native counterparts, as some of the wild-type allergens undergo posttranslational modifications. As it was shown for house dust mite chitinase -allergen, the protein purified from homogenized Dermatophagoides farinae mite bodies (*Der f 18*) bound IgE in 54% of the sera from patients with Dermatophagoides farinae allergy. The mature molecule amino acid sequence contains a single N-glycosylation site.[23,24]

The genetic engineering enabled modifications of the structure and subsequently the fate and the function of the gene products. The recombinant allergen proteins can present the reduced allergenic activity, increased immunogenicity or both features, compared with their natural counterparts.[17]

The genetic manipulations on allergens and their single epitopes opens new prospects for scientific research on immune response as well as for clinical diagnostics and treatment of immune-mediated diseases.

In the variety of allergen sources only some sequences or sections (epitopes) are responsible for their immunogenicity and allergenicity. The concept and terms of recombinant allergen-based component-resolved diagnostics (CRD) and immunotherapy (CRIT) was created in 1999 by Valenta. This approach advocates the use of well-defined allergens (components), for diagnosis of IgE-mediated allergy. Accordingly, application of the component-resolved immunotherapy is aimed to treat the patient with the clinically relevant allergens from a specific source only.[25]

Clinical Applications of Recombinant Allergens in Allergy Diagnostics

Currently, commercially available serum IgE binding tests contain purified or recombinant allergens. The recombinant allergens based in vitro assays give brand new opportunities to search for the particular epitopes of the clinical relevance in the allergic patient diagnostics as well to search for the mechanistic

of the allergen specific immune response. While the IgE binding to certain allergenic proteins can be predictive for allergic disease, some of the allergens, like cross-reactive profilins or certain glycan structures, are only weakly associated with clinical reactivity. As it was shown for apple allergens (*Mal d*) or hazelnut allergens (*Cor a*) hypersensitivity, presence of IgE specific to certain epitopes may correlate with the clinical severity of the allergic reactions. Noteworthy, the reactions can be geographically differentiated.[26] In some cases hypersensitivity to one single epitope can be responsible for the majority of allergic reactions, like in cat-dander (*Felis domesticus*) major allergen protein 1 (*Fel d 1*), allergy to birch *Bet v 1* or mugwort (*Artemisia vulgaris*) sole main allergen number 1 *Art v 1*.[27-29] CRD might result than in precised and targeted immunotherapy in such patients. Unfortunately, most of the common allergenic sources is much more reach in immunoreactive proteins, like in pollens or house dust mites. Grass pollen has 11 different allergens identified, some of them occur also as different isoforms and only some of these allergens have major general importance in grass pollen sensitization prevalence. The group 1 and 5 allergens of *Phleum pratense* are strong candidates for inclusion in a therapeutic vaccine.[17,30] The house dust mites contain above 30 allergenic proteins inducing specific IgE in the house dust mite allergic patients.[31]

Another important feature of the CRD is possibility of differentiation between the true allergen hypersensitivity (co-allergy) and the cross-reactivity between certain epitopes that, originate from even seemingly unrelated allergens. The cross-reactivity between epitopes results from their structural similarity and may induce the cross-binding of the IgE and allergic response in a patient primarily non-sensitized to a certain allergenic protein. The allergen cross reactions are an often observed phenomenon in patients with allergy. Pollen-food syndromes have been describedn[32] latex-vebetable or latex-fruit syndromes[33,34] as well as cross sensitizations to house dust mite species and other invertebrates allergens, and many other.[35] Some of the allergens (or epitopes) are common between species, families or even kingdoms. Homologous molecules of the birch pollen major allergen *Bet v1* can be found in pollen of evolutionary related *Fagales.*

Trees, like alder *Aln g 1*, hornbeam *Car b 1*, chestnut *Cas s 1*, hazel *Cor a 1*, beech *Fag s 1*, oak *Que a 1* and *Apiaceae* vegetables (e.g., celery *Api g 1*, carrot *Dau c 1*), as well as in *Rosaceae* fruits, like apple *Mal d 1*, cherry *Pru av 1*, apricot *Pru ar 1*, pear *Pyr c 1*, legumes, nuts, and seeds (e.g., hazelnut *Cor a 1*, soybean *Gly m 4*, peanut *Ara h 8*).[36] So far, 28 major groups of cross-reactive proteins have been identified: 6 groups of pathogenesis-related proteins, 11 groups of various enzymes (e.g., proteases, glycolytic enzymes, etc.) and others, such as transport proteins, protease inhibitors, regulatory or structural proteins.[36]

The most ubiquitous allergens are called panallergens. Known panallergens presently comprise only a few protein families, including profilins, polcalcins and non-specific lipid transfer proteins (nsLTP).[36]

The differential diagnostics of the hypersensitivity response ethiology is essential to apply the most efficient prophylaxis and treatment in the patient.[37,38]

The use of microarray tests allows a parallel analysis of IgE binding to large numbers of single allergens or peptides,[39] however the technique has also evident limitations, as some allergenic sources (almond, walnut) or some important allergens in certain allergen sources are still missing, e.g., peanut *Ara h 6* antigen. However, the large amount of data from a single microarray test becomes a challenge for interpretation in the terms of its clinical significance.[26]

Refinement of IgE-based testing may help elucidate the correlation or lack of correlation between allergenic sensitization and allergic disease, however the practical use and selection of allergenic components need to be evaluated in large studies including well-characterized allergic patients and healthy, non-sensitized controls and with representation of different geographical regions.[37] Selected clinical trials (completed and ongoing) with wild-type allergen or hypoallergenic derivative vaccines are listed in **Table 1**.

Recombinant Allergen Vaccines in Clinical Studies

The first clinical studies with recombinant allergens have delivered very encouraging results.[15] DNA recombination technology opened new prospects to generate allergen derivatives with reduced IgE-reactivity, hypoallergenic, with reduced risk of triggering undesirable allergic reactions during the course of immunotherapy, but with retained immunogenic vaccine activity. However numerous allergens have been cloned for research purposes, only a few have been applied in clinical studies.

Allergenic molecules applied in the clinical studies either retain the wild-type allergen structure or are modified. Clinical trials with the recombinant hypoallergens showed reduced IgE-mediated side effects or even lacked these effects during the allergen-specific immunotherapy. On the other hand, there could be observed the late-phase side effects, most likely resulting from the activation of allergen-specific T cells, as the hypoallergenic molecules possess a decreased allergen-specific IgE-binding capacity or do not bind the IgE at all.[16,40] Moreover, different vaccination routes are subjected to estimation and comparison in the studies. Currently, the clinical application of recombinant allergens, however promising, is not yet well established.

A double-blind placebo-controlled (DBPC) clinical trial performed by Jutel et al. in 2005 evaluated the use of mixture of five different wild-type recombinant allergens of timothy grass *Phl p* 1, *Phl p* 2, *Phl p* 5a, *Phl p* 5b and *Phl p* 6 in the treatment of periodic allergic rhinitis. The study was undertaken in 62 grass pollen allergic patients suffering from rhinoconjunctivitis with or without asthma and for the first time the clinical efficacy of a recombinant vaccine was reported.[17] Adsorbates of the five allergens were combined in approximately equimolar amounts and supplied in 3 dilutions. The highest concentration (strength 3) contained 50 μg/mL total protein. The initial dose contained 0.02 μg total protein. The dose was increased to 0.16 μg in the second injection and then doubled at subsequent injections to a maximum of 40 μg total protein (0.8 mL) 10 μg *Phl p* 1 (= 0.38 nmoles), 5 μg *Phl p* 2 (= 0.48 nmoles), 10 μg *Phl p* 5a (= 0.35 nmoles), 10 μg *Phl p* 5b (= 0.38 nmoles), and 5 μg

Table 1. Immunotherapy trials with indication of their phase, clinicalTrials.gov ID and route of administration of recombinant wild-type allergen and hypoallergen vaccines

Vaccine	Target	Specific proteins	Route of administration	Trial design	Summary of results of the trial	ClinicalTrials.gov ID or/and literature references
Recombinant wild-type allergens	Individuals with seasonal rhinoconjunctivitis and/or mild asthma	Birch pollen/Bet v 1	SCIT	DBPC, phase II, safety and efficacy	Positive	NCT00410930[47]
	Individuals with birch pollen induced allergic rhinitis		SLIT	DBPC, phase I, safety, tolerability and pharmacodynamic effects	No publications provided	NCT00396149
			SLIT	phase I, safety, tolerability and pharmacodynamic effects	Positive	NCT00889460[48]
	Individuals with birch pollen-related allergic rhinoconjunctivitis		SLIT	DBPC, phase II, safety and efficacy	No publications provided	NCT00901914
	Individuals with allergic rhinoconjunctivitis, with or without asthma	Grass pollen/Phl p 1, Phl p 2, Phl p 5a, Phl p 5b, Phl p 6	SCIT	DBPC, phase III, safety and efficacy	No publications provided	NCT00309036
			SCIT	DBPC, phase III, safety and efficacy	No publications provided	NCT00671268
			SCIT	DBPC, phase II, safety and efficacy, dose response	No publications provided	NCT00666341
			SCIT	DBPC, phase III, safety and efficacy	No publications provided	NCT01353755
			SCIT	DBPC	Positive	[17]
	Peanut-allergic individuals	Peanut/Ara h 1, Ara h 2, Ara h 3	rectal	phase I	Positive	NCT00850668
Hypoallergens	Individuals allergic to birch pollen allergens	Birch pollen/Bet v 1 folding variant	SCIT	OC, phase II, safety and efficacy	No publications provided	NCT00266526
			SCIT	DBPC, phase II, immunological and histological evaluation	No publications provided	NCT00841516
			SCIT	DBPC	Positive	[40, 42, 43, 44, 45, 46]
	Individuals with allergic rhinoconjunctivitis		SCIT	DBPC, phase III, safety and efficacy	No publications provided	NCT00309062
			SCIT	DBPC, phase III, safety and efficacy	No publications provided	NCT00554983

DBPC, double blind placebo-controlled; OC, open controlled; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; NCT, clinicalTrials.gov ID (based on National Institutes of Health Clinical trial database - http://clinicaltrials.gov and[49,72]).

Phl p 6 (= 0.42 nmoles), together with 1 mg/mL Al31 in physiological saline. The vaccine was administered subcutaneously for 18 mo. A combined symptom–medication score (SMS) adopted as the primary end-point showed a 39% improvement in the active treatment group, relative to placebo ($p < 0.041$). Active treatment induced highly significant increases in both IgG1 and IgG4 grass pollen specific antibody concentrations together with a significant decrease in IgE. Specific IgE levels were not significantly different between groups at the beginning of the study, but thereafter the active treatment group showed a downward trend with values significantly lower than the baseline. The treatment related adverse events were observed in association with 78

Table 1. Immunotherapy trials with indication of their phase, clinicalTrials.gov ID and route of administration of recombinant wild-type allergen and hypoallergen vaccines

Vaccine	Target	Specific proteins	Route of administration	Trial design	Summary of results of the trial	ClinicalTrials.gov ID or/and literature references
Recombinant wild-type allergens	Individuals with seasonal rhinoconjunctivitis and/or mild asthma	Birch pollen/Bet v 1	SCIT	DBPC, phase II, safety and efficacy	Positive	NCT00410930 [47]
	Individuals with birch pollen induced allergic rhinitis		SLIT	DBPC, phase I, safety, tolerability and pharmacodynamic effects	No publications provided	NCT00396149
			SLIT	phase I, safety, tolerability and pharmacodynamic effects	Positive	NCT00889460 [48]
	Individuals with birch pollen-related allergic rhinoconjunctivitis		SLIT	DBPC, phase II, safety and efficacy	No publications provided	NCT00901914
	Individuals with allergic rhinoconjunctivitis, with or without asthma	Grass pollen/Phl p 1, Phl p 2, Phl p 5a, Phl p 5b, Phl p 6	SCIT	DBPC, phase III, safety and efficacy	No publications provided	NCT00309036
			SCIT	DBPC, phase III, safety and efficacy	No publications provided	NCT00671268
			SCIT	DBPC, phase II, safety and efficacy, dose response	No publications provided	NCT00666341
			SCIT	DBPC, phase III, safety and efficacy	No publications provided	NCT01353755
			SCIT	DBPC	Positive	[17]
	Peanut-allergic individuals	Peanut/Ara h 1, Ara h 2, Ara h 3	rectal	phase I	Positive	NCT00850668
Hypoallergens	Individuals allergic to birch pollen allergens	Birch pollen/Bet v 1 folding variant	SCIT	OC, phase II, safety and efficacy	No publications provided	NCT00266526
			SCIT	DBPC, phase II, immunological and histological evaluation	No publications provided	NCT00841516
			SCIT	DBPC	Positive	[40], [42], [43], [44], [45], [46]
	Individuals with allergic rhinoconjunctivitis		SCIT	DBPC, phase III, safety and efficacy	No publications provided	NCT00309062
			SCIT	DBPC, phase III, safety and efficacy	No publications provided	NCT00554983

DBPC, double blind placebo-controlled; OC, open controlled; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; NCT, clinicalTrials.gov ID (based on National Institutes of Health Clinical trial database - http://clinicaltrials.gov and[49,72]).

injections (10.7%) in the active treatment group and 44 injections (5.9%) in the placebo group. All the subjects continued treatment without further problems and it was concluded that the preparation showed a favorable safety when compared with findings from other immunotherapy studies.

Linhart et al.[41] presented in 2005 results of vaccination with hybrid molecule engineered by expression of the cDNA coding the 4 major grass pollen allergens and containing most of the B-cell epitopes of grass pollen. The molecule could be used to diagnose allergy in 98% (n = 652) of patients allergic to grass pollen.

A clinical study performed to compare the allergenic activity of wild-type *rBet v 1* with recombinant *Bet v 1* derivatives (*rBet v 1* fragments, dimer and trimer) with potentially reduced anaphylactic activity showed that the genetically modified hypoallergenic derivatives of the major birch pollen allergen present reduced capacity to induce immediate type skin reactions (Pauli et al., 2000).[42]

In the first DBPC immunotherapy study with recombinant allergen preparations, 2 different hypoallergenic derivatives of the major birch pollen allergen *Bet v 1* and placebo were compared by Niederberger and colleagues (2004). The hypoallergenic rBet v 1 derivatives adsorbed to aluminum hydroxide were formulated: two recombinant Bet v 1 (rBet v 1) fragments and a rBet v 1 trimer. The vaccines contained either an equimolar mixture of the two fragments or a trimer (100 g of protein per ml of adsorbate), or aluminum hydroxide alone (placebo). Patients received typically eight s.c. injections containing increasing doses (1, 2, 4, 8, 10, 20, 40 and 80 μg of protein) of the trial preparations or placebo in one to two weekly intervals as a preseasonal treatment. Because of the strongly reduced allergenic activity of the recombinant allergen derivatives, maximal doses of 80 μg of the active preparations per injection were tolerated by most of the patients. As reported, the active treatment induced protective IgG antibodies that inhibited allergen-induced release of inflammatory mediators. A reduction of cutaneous sensitivity as well as an improvement of symptoms in actively treated patients was observed and, most important, rises of allergen-specific IgE induced by seasonal birch pollen exposure were significantly reduced in vaccinated patients.[43] In another DBPC study, genetically modified derivatives of *Bet v 1* (*Bet v 1*-trimer, *Bet v 1*-fragments) were applied. In the study, subcutaneous injections of aluminum hydroxide–adsorbed allergen derivatives containing increasing doses (1–80 μg) of recombinant Bet v 1 derivatives or placebo were administered. Vaccination with genetically modified *Bet v 1* derivatives, but not with placebo, induced *Bet v 1*-specific IgG1, IgG2 and IgG4 and low IgA antibodies in serum, which also appeared in nasal secretions. The levels of therapy-induced *Bet v 1*-specific IgG4 antibodies in nasal secretions were significantly ($p < 0.05$) associated with reduced nasal sensitivity to natural, birch pollen-derived *Bet v 1* as objectively determined by controlled nasal provocation experiments (Reisinger et al., 2005).[44]

As shown in another study by Niederberger et al. (2007), the use of *Bet v 1* fragments or *Bet v 1* trimers increased IgG1, IgG2 and IgG4 specific to cross-reactive allergens, like alder pollen, hazel pollen, celery, carrot, and apple, with subsequent improvement in oral allergy syndrome symptoms mainly in the *verum* group patients. Actively treated patients had received a cumulative injected dose of 150.0 μg recombinant protein on average (range, 4–245 μg).[45]

Pree et al.,[46] demonstrated in 2007 that vaccination with folded and unfolded recombinant allergen derivatives of *Bet v 1* induces IgG against new epitopes. The birch pollen allergic patients received vaccinations with aluminum hydroxide-adsorbed derivatives of Bet v 1 (an equimolar mixture of two hypoallergenic Bet v 1 fragments, hypoallergenic Bet v 1 trimer, or aluminum hydroxide alone (placebo). Patients treated with Bet v 1 trimer received on average 8.4 injections (range: 7–10) and an average cumulative injected dose of 171 μg (range: 69–265 μg) of the active preparation. Patients receiving Bet v 1 fragments were given an average injection course of 8.5 injections (range: 7–9) and an average cumulative dose of 168 μg (range: 85–245 μg). The *Bet v 1* trimers retained more of the folded configuration of natural *Bet v 1,* compared with *Bet v 1* fragments, and the trimers increase the production of *Bet v 1* IgE more than the fragments.[38]

A multicenter, randomized, DBPC recombinant allergen study, conducted by Pauli and colleagues (2008) compared a recombinant birch pollen allergen vaccine, standard birch pollen extract, natural purified birch pollen allergen and aluminum hydroxide as placebo in 134 patients with birch allergy. The patients were immunized subcutaneously over 2 y. All 3 verum groups demonstrated significant and equal improvement in symptoms, medication use and skin test reactivity in both pollen seasons compared with the placebo group. Interestingly, the *rBet v*–treated individuals presented a greater increase in *Bet v 1* specific IgG1, IgG2 and IgG4 levels as well as a greater decrease in the skin test reactivity than the other verum groups. However, new sensitizations were reported in the SIT-undergoing patients.[47]

As concluded by Purohit et al., 2008 in a three-arm DBPC study with a recombinant *Bet v 1* trimer and an equimolar mixture of two recombinant *Bet v 1* fragments together, single courses of injection immunotherapy with *Bet v 1* allergen derivatives showed trends toward improved well-being and reduced reactivity to specific allergen provocation, but did not yield significant improvement in the combined SMS. Therapy with the trimer preparation was associated with more local side effects, whereas the *Bet v 1* fragments were more likely to induce systemic reactions. The vaccine dosage was increased from 1 to 80 μg in 2-fold concentration steps with subcutaneous injections given at 7–14 d intervals.[40]

Clinical studies with r*Bet v 1* tablets for sublingual allergen specific immunotherapy (SLIT) has also been initiated, as reported by Winther (2009). In this study, patients were randomized to receive rBet v 1 SLIT (at doses from 12.5 μg to 300 μg) or placebo for 2 weeks.[48]

The food allergy specific immunotherapy is also under investigation. There is reported a phase I clinical study of recombinant modified peanut allergens (*Ara h 1, Ara h 2, Ara h 3*), encapsulated in heat/phenol-killed *E. coli,* designed for rectal immunotherapy, in the National Institutes of Health's clinical trial database. [16]

Peptide Vaccines Clinical Studies

The peptide vaccines have been applied in cat allergy (*Fel d 1* peptides), in bee-venom allergy (*Api m 1* peptides) and in ragweed allergy (published in an abstract only) clinical studies.[50,51]

Lack of the secondary structure in the peptide epitopes results in the lack of allergenicity of these molecules. The non-allergenic peptides are also able to induce IgG-mediated immune response due to chemical coupling to other proteins, like highly repetitive antigens in bacteria and viruses, thus enhancing the allergen-specific immunotherapy efficiency.[52] On the other hand, the constructs may be responsible for new immune-related side effects of SIT.[53]

Simon and colleagues (1996) tested peptide immunotherapy in cat-dander allergic humans, using a formation of two synthetic peptides, IPC-1 and IPC-2, each of which is 27 amino acids long, containing T cell-reactive regions of *Fel d 1,* in a randomized, double-blind, parallel-group study. Forty-two subjects received subcutaneous injections of treatment peptides (250 μg) or placebo weekly for four consecutive weeks. Changes in immediate- and late-phase skin test reactivity and in antigen-driven cytokine synthesis were assessed. Epicutaneous (end-point titration) and intradermal tests were performed with cat extract containing *Fel d 1*, before the first injection, then 2, 6 and 24 weeks after the fourth and last injection of peptides or placebo. IL-4, IL-10 and IFN-γ expression by circulating peripheral blood mononuclear cells (PBMC) in response to the cat allergen extract was measured. As concluded, the peptide immunotherapy neither reduced the immediate- or late-phase skin reactivity to the *Fel d 1* containing cat allergen extract nor modified cat antigen-specific cytokine production significantly.[54]

The effects of immunotherapy with *Fel d 1* peptides on the response to bronchial provocation tests with a standardized *Fel d 1* cat allergen extract on *Fel d 1*-specific serum IgE and IgG levels and in vitro IL-4 and IFN-γ production was investigated by Pene et al. (1998). Patients allergic to cats received 6 weekly injections of 7.5 μg (low dose), 75 μg (medium dose), or 750 μg (high dose) of *Fel d 1* peptides (25 patients) or a placebo (6 patients). In this study, the IL-4 release was significantly reduced in the high dose-treated group ($p < 0.005$), whereas it was unchanged in the low or medium dose- and in the placebo-treated groups. In all groups, IFN-γ, IgE, and IgG levels remained unchanged.

Norman and colleagues (1996) induced in allergic humans the counterpart of murine experimental T-cell tolerance. T-cell lines from cat-allergic humans were used to map T-cell epitopes for *Fel d 1*. Two peptides of 27 amino acids each were synthesized to contain the dominant epitopes. Ninety-five cat-sensitive patients were randomized into placebo, 7.5, 75 and 750 μg groups. As concluded, the T-cell reactive treatment peptides safely improved allergic responses to cats. Linear trend analysis indicated a significant dose response effect: $p = 0.05$ for nose and 0.03 for lung symptoms. Allergic side effects occurred an hour or more after the first 750 μg dose in 16 of 24 patients but required little or no treatment with one exception.[55]

In a multicenter, randomized, DBPC study of 133 cat allergic patients chronically exposed to cats or who had failed previous conventional cat immunotherapy synthetic cat allergen peptides (IPC-1 and IPC-2, ALLERVAX CAT) were generated and administered to the patients subcutaneously, in doses 75 or 750 μg of each peptide (Maguire, 1999). Most of adverse events were late allergy responses, commonly associated with respiratory symptoms, and these events declined with successive injections. However, the vaccination improved tolerance to cats and improved pulmonary function in cat allergic patients with reduced FEV1.[56]

Intradermal administration of short overlapping peptides derived from chain 1 of the cat allergen *Fel d 1* (FC1P) (40 μg) that did not cross-link IgE, elicited isolated late asthmatic reactions with no visible early or late cutaneous response in 9/40 cat-allergic asthmatics, as showed by Haselden and colleagues (1999). They conclude that short, allergen-derived peptides can directly initiate a major histocompatibility complex-restricted T cell-dependent late asthmatic reaction, without the requirement for an early IgE/mast cell-dependent response in sensitized asthmatic subjects.[57] The same author confirm that the asthma process evoke during peptide vaccination in cat dander-allergic patients might involve T cell-dependent airway narrowing with no requirement for IgE, mast cells or infiltrating inflammatory cells. A randomized, placebo-controlled, crossover study involved bronchial and skin biopsies and bronchoalveolar lavage (BAL) fluids from 8 cat-allergic subjects who developed significant late asthmatic reactions 6 h after intradermal injection of 80 μg of Fel d 1 chain 1-derived peptides (FC1Ps). Immunostaining of bronchial biopsy specimens showed no changes in the numbers of eosinophils, neutrophils, basophils, mast cells, CD3(+), CD4(+) or CD8(+) T cells, CD25(+) cells or macrophages, or cells mRNA(+) for IL-4, IL-5 or IL-13 when the FC1P day was compared with the diluent control day. There were also no significant differences in eosinophil numbers, either in BAL fluids or in peripheral blood after FC1P challenge. Furthermore, there were no significant alterations in the concentrations of histamine, histamine-releasing factors, or eicosanoids [LTC(4)/D(4)/E(4), PGD(2), PGE(2), TXB(2), PGF(2α)] in BAL fluids. FC1Ps induced a significant ($p < 0.05$) elevation in CD8(+) cells in the skin and an unexpected decrease in IL-5 in BAL fluids ($p = 0.043$).[58]

Allergen-derived peptides induce tolerance to subsequent peptide injection in the target organ - the lung, reduce late-phase cutaneous responsiveness to whole allergen and alter in vitro T cell reactivity. As it was shown, a second injection of cat allergen (*Fel d 1*)-derived T cell peptides (1, 2.5 or 5 μg, in three patient groups, respectively) was associated with a marked reduction or absence, of the late allergic reaction (LAR) and that up to 40 weeks was required for return to baseline values. The cutaneous late-phase reaction to whole cat dander was also inhibited, even in subjects who did not experience an initial LAR. Significant decrease in peptide- and whole allergen-induced proliferation of PBMCs and the production of IL-4, IL-13 and IFN-γ in cultures.[59]

The effect of multiple injections with short overlapping T-cell peptides derived from *Fel d 1,* (90 μg in increasing divided doses) on the magnitude of the early and late phase skin reactions to intact allergens was confirmed in another randomized, placebo-controlled study of Oldfield and colleagues (2002). Patients in the peptide group but not the placebo group had a significant reduction in the size of their late reaction to whole cat dander measured at baseline and two follow-up periods: 4–8 weeks and 3–9 mo. The size of the early reaction to *Fel d 1*, but not to whole cat dander was significantly reduced in patients receiving peptides compared with those on placebo. Patients on peptide treatment had a significantly greater decrease in the concentration of interferon gamma and interleukin 13 and in the amount of proliferation between baseline and first follow-up than did those on placebo, the concentration of interferon gamma and of interleukin 4 and 13 and the cell proliferation significantly decreased between the baseline and the second follow-up, and the concentration of interleukin 10 was significantly higher in

peptides treated patients, however, none of these values differed significantly between groups.[60]

To evaluate the effect of T cell peptide therapy on the allergen-induced cutaneous late-phase reaction, the allergen-induced, late-phase skin biopsies before and after T cell peptide therapy for evidence of alterations in the pattern of local recruitment of Th1, T-helper type 2 (Th2) and T regulatory cells were studied (Alexander, 2005). Treatment with allergen-derived T cell peptides (incremental doses of Fel d 1 peptides—0.1, 1, 5, 10 and 25 mg; amount of each peptide in an 11-peptide mixture) resulted in allergen-dependent recruitment to the skin of CD4+/IFN-γ+ ($p = 0.03$) and CD4+/CD25+ cells (concluded as Th1 profile), but not in CD4+/IL-10+ or CD4+/CTLA-4+ cells (regarded as Th2/T regulatory cells), to cutaneous late-phase reaction sites.[61] The same author performed a pilot study to determine whether overlapping *Fel d 1*-derived T-cell peptides treatment affects allergen-induced nasal and bronchial reactions, as well as asthma/rhinitis quality of life (QOL). However the treatment (approximately 300 μg in increasing, divided doses) appeared to have potential for inhibiting upper and lower airway outcome measurements in cat allergic patients, as concluded, larger, dose-ranging, studies are required to confirm the clinical efficacy of peptide allergen therapy.[62]

Specific immunotherapy with honeybee venom, however highly effective, carries a risk of serious adverse events during the treatment. The study of Mueller et al. (1998) investigated the immunologic mechanisms and clinical effects of immunotherapy with T-cell epitope peptides of the major bee venom allergen, the phospholipase A2 (PLA) in SIT of 5 bee venom-allergic individuals. As reported, no allergic side effects were caused by the peptide immunotherapy, administered in increasing doses, up to a maintenance dose of 100 μg. The subsequent allergen (bee sting) challenge with PLA was without systemic allergic symptoms. The production of TH2 and TH1 cytokines was inhibited, while B cells were not affected in their capacity to produce specific IgE and IgG4 antibodies, in a fashion similar to that of conventional immunotherapy in successfully treated patients.[63] To evaluate the safety and immunogenicity of an allergen-derived long synthetic overlapping peptide (LSP) immunotherapy, a DBPC phase I clinical trial in patients hypersensitive to bee venom was performed by Fellrath and colleagues (2003). The peptides were administered in a dose-escalating protocol to a maintenance dose of 100 μg per a peptide. The immunotherapy was safe and able to induce Th1-type immune deviation (IFN-gamma secretion assessed), allergen-specific IL-10 production, and T-cell hyporesponsiveness.[64] Also in an open, controlled study of Tarzi et al. (2006), the treatment with an HLA-DR-based PLA(2) peptide vaccine (TCR epitope) in subjects with mild honeybee allergy was assessed. Treated volunteers received nine incremental doses of peptide mixture at weekly intervals to a cumulative dose of 431.1 mg of each peptide, administered by the intradermal route as follows: 0.1, 1, 5, 25, 50, 50, 100, 100 and 100 μg. The safety and effectiveness of the peptide vaccine was reported. Proliferation of venom-stimulated PBMCs decreased in treated subjects compared with controls ($p = 0.01$). Peptide treatment reduced the production of IL-13 by PLA(2)-stimulated PBMCs ($p < 0.01$) and IFN-γ ($p < 0.01$), and increased the production of IL-10 ($p = 0.02$). A transient, but modest, increase in allergen-specific IgG was also observed.[65]

Another Vanguard Vaccination Approaches in Clinical and Preclinical Studies

Attempts have been undertaken to modify recombinant allergens in order to improve therapeutic efficacy in SIT while reducing allergic side-effects using modular antigen-translocation (MAT) technology, for intracellular targeting of allergens to the major histocompatibility class-II (MHC-II) presentation pathway to enhance antigen presentation, or vaccines consisting of nonallergenic peptides fused to carriers.[66]

Martin, Gomez et al. applied the major cat allergen *Fel d 1* fused to a TAT-derived protein translocation domain and to a truncated invariant chain for targeting the MHC class II pathway (MAT-Fel d 1). The immunogenicity of such constructs was assessed in mice. The mice were immunized thrice with 30 pmol intralymphatically (i.l.) or 300 pmol s.c. recombinant Fel d 1, TAT-Fel d 1 or MAT-Fel d 1. The potential safety was estimated by cellular antigen stimulation test (CAST) using basophils from cat-dander-allergic patients. The MAT-*Fel d 1* allergen enhanced protective antibody and Th1 responses in mice, while reducing human basophil degranulation. Immunotherapy using MAT-*Fel d 1* allergen showed the potential to enhance SIT efficacy and safety and allows shortening the SIT.[67] Zaleska et al. showed results of MAT-*Fel d 1* vaccine, applied intranodally. MAT-Fel d 1 vaccine adsorbed to alum was administered by 3 intralymphatic injections in increasing dose (1, 3 and 10 μg) into inguinal, subcutaneous lymph node within 2 mo with 4 weeks intervals. The *Fel d 1*-specific T-cell tolerance was maintained in the treated group, compared with the placebo group yet after 12 mo after the therapy had stopped. Relative changes in the level of specific IgE in serum of treated patients measured by ELISA were contrary to the lack of drug related side effects and to the improvement of allergen tolerance in nasal provocation test.[68]

Focke and colleagues reported an approach for the rational design of B cell epitope-derived peptide allergy vaccines to avoid a secondary, specific treatment-related sensitization in the patients. Accordingly, the three-dimensional (3-D) structure of birch pollen allergen *Bet v 1*, six peptides comprising 25–32 preferably solvent-exposed amino acids were synthesized, lacking the IgE-inducing secondary structure. The in vivo allergenic activity of the peptides was studied by SPT in five birch pollen-allergic patients and a grass pollen allergic patient without birch pollen allergy. The peptide-based allergy vaccine was evaluated in a mouse model for birch pollen allergy. Prophylactic vaccination was performed with a mixture of the six peptides, 5 μg each. As concluded, the results indicate the mechanistic importance of blocking antibodies for allergy vaccination.[69]

Other new approach, applied in mice, was changing the IgE-binding epitopes of *Fel d 1* without disrupting T-cell epitopes, by inserting duplications of T-cell epitopes (DTE) on both polypeptide chains of *Fel d 1* and by disrupting disulphide bonds linking the two chains. The hypoallergen with the most reduced IgE

reactivity, rFel d 1 (DTE III) was selected for the vaccination. Groups of sensitized mice were therapeutically treated (s.c.) with 50 μg rFel d 1, 50 or 200 μg rFel d 1 (DTEIII) in phosphate buffered saline (PBS), or PBS only (sham treatment). Mice from the *verum* groups produced increased serum levels of rFel d 1-specific IgG1 and IgG2a compared with the sham-treated mice. The 200 μg rFel d 1 (DTE III) treatment tended to reduce airway hyperresponsiveness. All mice tolerated treatment with rFel d 1 (DTE III), in contrast to only four of ten treated with rFel d 1. Compared with rFel d 1, the hypoallergen showed a tendency of reduced SPT reactivity.[70]

Niespodziana et al. reports the results of a pre-clinical study of cat allergy specific immunotherapy with fused proteins of the hepatitis B virus-derived PreS domain and 2 nonallergenic *Fel d 1*-derived peptides, expressed in *E. coli*. The recombinant fusion proteins contained less than 40% of the *Fel d 1* sequence and exhibited not only strongly reduced IgE-mediated immune response, but also were lacking many of the specific T-cell epitopes.[71]

Conclusions

Several forms of recombinant vaccines for S IT have been approached inclincal trials. They include wild-type allergens or molecules with reduced allergenic activity, increased immunogenicity, or both. In additioon, peptide fragments of corresponding T-cell epitopes of the specific allergens to induce immunologic tolerance and decrease allergenicity are under clinical invesigation. Current clinical data with these approaches are very promising. However, this new technology demands an individual approach to the patients. In the mono-sensitized patients standard cocktails of relevant major allergens provide optimal cure. In the polisensitized subjects component resolved approach could be more effective. However, although there are a number of very successful phase II clinical trials the major problem to overcome is the of the very demandingphase III studies, which are necessary before the treatment can be offered as the clinical routine.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Pajno GB, Barberio G, De Luca F, Morabito L, Parmiani S. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. Clin Exp Allergy 2001; 31:1392-7; PMID:11591189; http://dx.doi.org/10.1046/j.1365-2222.2001.01161.x.
2. Jacobsen L, Niggemann B, Dreborg S, Ferdousi HA, Halken S, Høst A, et al.; (The PAT investigator group). Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. Allergy 2007; 62:943-8; PMID:17620073; http://dx.doi.org/10.1111/j.1398-9995.2007.01451.x.
3. Jutel M, Akdis CA. Immunological mechanisms of allergen-specific immunotherapy. Allergy 2011; 66:725-32; PMID:21466562; http://dx.doi.org/10.1111/j.1398-9995.2011.02589.x.
4. Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy. J Allergy Clin Immunol 2011; 127:18-27, quiz 28-9; PMID:21211639; http://dx.doi.org/10.1016/j.jaci.2010.11.030.
5. Noon L. Prophylactic inoculation against hay fever. Lancet 1911; 177:1572-3; http://dx.doi.org/10.1016/S0140-6736(00)78276-6.
6. Ring J, Gutermuth J. 100 years of hyposensitization: history of allergen-specific immunotherapy (ASIT). Allergy 2011; 66:713-24; PMID:21320133; http://dx.doi.org/10.1111/j.1398-9995.2010.02541.x.
7. Grönlund H, Vrtala S, Wiedermann U, Dekan G, Kraft D, Valenta R, et al. Carbohydrate-based particles: a new adjuvant for allergen-specific immunotherapy. Immunology 2002; 107:523-9; PMID:12460198; http://dx.doi.org/10.1046/j.1365-2567.2002.01535.x.
8. Ibarrola I, Sanz ML, Gamboa PM, Mir A, Benahmed D, Ferrer A, et al. Biological characterization of glutaraldehyde-modified Parietaria judaica pollen extracts. Clin Exp Allergy 2004; 34:303-9; PMID:14987312; http://dx.doi.org/10.1111/j.1365-2222.2004.01859.x.
9. Marsh DG, Norman PS, Roebber M, Lichtenstein LM. Studies on allergoids from naturally occurring allergens. III. Preparation of ragweed pollen allergoids by aldehyde modification in two steps. J Allergy Clin Immunol 1981; 68:449-59; PMID:6171586; http://dx.doi.org/10.1016/0091-6749(81)90199-8.
10. Cox L. Standardized allergen extracts: past, present and future. Expert Rev Clin Immunol 2005; 1:579-88; PMID:20477599; http://dx.doi.org/10.1586/1744666X.1.4.579.
11. Aalberse RC, Crameri R. IgE-binding epitopes: a reappraisal. Allergy 2011; 66:1261-74; PMID:21623828; http://dx.doi.org/10.1111/j.1398-9995.2011.02656.x.
12. Casale TB, Stokes JR. Future forms of immunotherapy. J Allergy Clin Immunol 2011; 127:8-15, quiz 16-7; PMID:21094518; http://dx.doi.org/10.1016/j.jaci.2010.10.034.
13. Bohle B, Breitwieser A, Zwölfer B, Jahn-Schmid B, Sára M, Sleytr UB, et al. A novel approach to specific allergy treatment: the recombinant fusion protein of a bacterial cell surface (S-layer) protein and the major birch pollen allergen Bet v 1 (rSbsC-Bet v 1) combines reduced allergenicity with immunomodulating capacity. J Immunol 2004; 172:6642-8; PMID:15153479.
14. Moldaver D, Larché M. Immunotherapy with peptides. Allergy 2011; 66:784-91; PMID:21507007; http://dx.doi.org/10.1111/j.1398-9995.2011.02610.x.
15. Zeiler T, Taivainen A, Rytkönen M, Rautiainen J, Karjalainen H, Mäntyjärvi R, et al. Recombinant allergen fragments as candidate preparations for allergen immunotherapy. J Allergy Clin Immunol 1997; 100:721-7; PMID:9438477; http://dx.doi.org/10.1016/S0091-6749(97)70264-1.
16. Valenta R, Niespodziana K, Focke-Tejkl M, Marth K, Huber H, Neubauer A, et al. Recombinant allergens: what does the future hold? J Allergy Clin Immunol 2011; 127:860-4; PMID:21458656; http://dx.doi.org/10.1016/j.jaci.2011.02.016.
17. 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; 116:608-13; PMID:16159631; http://dx.doi.org/10.1016/j.jaci.2005.06.004.
18. Young RA, Davis RW. Yeast RNA polymerase II genes: isolation with antibody probes. Science 1983; 222:778-82; PMID:6356359; http://dx.doi.org/10.1126/science.6356359.
19. Thomas WR. The advent of recombinant allergens and allergen cloning. J Allergy Clin Immunol 2011; 127:855-9; PMID:21251702; http://dx.doi.org/10.1016/j.jaci.2010.12.1084.
20. 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; 167:175-82; PMID:3335830; http://dx.doi.org/10.1084/jem.167.1.175.
21. 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; 85:127-9; PMID:3276629; http://dx.doi.org/10.1159/000234488.
22. Fang KS, Vitale M, Fehlner P, King TP. cDNA cloning and primary structure of a white-face hornet venom allergen, antigen 5. Proc Natl Acad Sci U S A 1988; 85:895-9; PMID:3422469; http://dx.doi.org/10.1073/pnas.85.3.895.
23. Weber E, Hunter S, Stedman K, Dreitz S, Olivry T, Hillier A, et al. Identification, characterization, and cloning of a complementary DNA encoding a 60-kd house dust mite allergen (Der f 18) for human beings and dogs. J Allergy Clin Immunol 2003; 112:79-86; PMID:12847483; http://dx.doi.org/10.1067/mai.2003.1602.
24. Thomas WR, Hales BJ, Smith WA. House dust mite allergens in asthma and allergy. Trends Mol Med 2010; 16:321-8; PMID:20605742; http://dx.doi.org/10.1016/j.molmed.2010.04.008.
25. Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Grönlund H. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). Clin Exp Allergy 1999; 29:896-904; PMID:10383589; http://dx.doi.org/10.1046/j.1365-2222.1999.00653.x.
26. Knol EF, Knulst AC. Application of multiplexed immunoglobulin E determination on a chip in component-resolved diagnostics in allergy. Clin Exp Allergy 2010; 40:190-2; PMID:19912247; http://dx.doi.org/10.1111/j.1365-2222.2009.03402.x.
27. Uermösi C, Beerli RR, Bauer M, Manolova V, Dietmeier K, Buser RB, et al. Mechanisms of allergen-specific desensitization. J Allergy Clin Immunol 2010; 126:375-83; PMID:20624641; http://dx.doi.org/10.1016/j.jaci.2010.05.040.
28. Jahn-Schmid B, Sirven P, Leb V, Pickl WF, Fischer GF, Gadermaier G, et al. Characterization of HLA class II/peptide-TCR interactions of the immunodominant T cell epitope in Art v 1, the major mugwort pollen allergen. J Immunol 2008; 181:3636-42; PMID:18714038.
29. Ebner C, Szépfalusi Z, Ferreira F, Jilek A, Valenta R, Parronchi P, et al. Identification of multiple T cell epitopes on Bet v I, the major birch pollen allergen, using specific T cell clones and overlapping peptides. J Immunol 1993; 150:1047-54; PMID:7678615.

30. Westritschnig K, Horak F, Swoboda I, Balic N, Spitzauer S, Kundi M, et al. Different allergenic activity of grass pollen allergens revealed by skin testing. Eur J Clin Invest 2008; 38:260-7; PMID:18339006; http://dx.doi.org/10.1111/j.1365-2362.2008.01938.x.
31. Thomas WR, Smith WA, Hales BJ, Mills KL, O'Brien RM. Characterization and immunobiology of house dust mite allergens. Int Arch Allergy Immunol 2002; 129:1-18; PMID:12372994; http://dx.doi.org/10.1159/000065179.
32. Egger M, Mutschlechner S, Wopfner N, Gadermaier G, Briza P, Ferreira F. Pollen-food syndromes associated with weed pollinosis: an update from the molecular point of view. Allergy 2006; 61:461-76; PMID:16512809; http://dx.doi.org/10.1111/j.1398-9995.2006.00994.x.
33. Gamboa PM, Sánchez-Monge R, Díaz-Perales A, Salcedo G, Ansótegui J, Sanz ML. Latex-vegetable syndrome due to custard apple and aubergine: new variations of the hevein symphony. J Investig Allergol Clin Immunol 2005; 15:308-11; PMID:16433216.
34. Blanco C. Latex-fruit syndrome. Curr Allergy Asthma Rep 2003; 3:47-53; PMID:12542994; http://dx.doi.org/10.1007/s11882-003-0012-y.
35. Sidenius KE, Hallas TE, Poulsen LK, Mosbech H. Allergen cross-reactivity between house-dust mites and other invertebrates. Allergy 2001; 56:723-33; PMID:11488665; http://dx.doi.org/10.1034/j.1398-9995.2001.056008723.x.
36. Hauser M, Roulias A, Ferreira F, Egger M. Panallergens and their impact on the allergic patient. Allergy Asthma Clin Immunol 2010; 6:1; PMID:20298513; http://dx.doi.org/10.1186/1710-1492-6-1.
37. Skamstrup Hansen K, Poulsen LK. Component resolved testing for allergic sensitization. Curr Allergy Asthma Rep 2010; 10:340-8; PMID:20628838; http://dx.doi.org/10.1007/s11882-010-0133-z.
38. Weber RW. Cross-reactivity of pollen allergens: impact on allergen immunotherapy. Ann Allergy Asthma Immunol 2007; 99:203-11; quiz 12-3, 31.
39. Hiller R, Laffer S, Harwanegg C, Huber M, Schmidt WM, Twardosz A, et al. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. FASEB J 2002; 16:414-6; PMID:11790727.
40. Purohit A, Niederberger V, Kronqvist M, Horak F, Grönneberg R, Suck R, et al. Clinical effects of immunotherapy with genetically modified recombinant birch pollen Bet v 1 derivatives. Clin Exp Allergy 2008; 38:1514-25; PMID:18564326; http://dx.doi.org/10.1111/j.1365-2222.2008.03042.x.
41. 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; 115:1010-6; PMID:15867859; http://dx.doi.org/10.1016/j.jaci.2004.12.1142.
42. 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; 30:1076-84; PMID:10931114; http://dx.doi.org/10.1046/j.1365-2222.2000.00869.x.
43. 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; PMID:15310844; http://dx.doi.org/10.1073/pnas.0404735101.
44. Reisinger J, Horak F, Pauli G, van Hage M, Cromwell O, König 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; 116:347-54; PMID:16083789; http://dx.doi.org/10.1016/j.jaci.2005.04.003.
45. Niederberger V, Reisinger J, Valent P, Krauth MT, Pauli G, van Hage M, et al. Vaccination with genetically modified birch pollen allergens: immune and clinical effects on oral allergy syndrome. J Allergy Clin Immunol 2007; 119:1013-6; PMID:17292956; http://dx.doi.org/10.1016/j.jaci.2006.12.661.
46. Pree I, Reisinger J, Focke M, Vrtala S, Pauli G, van Hage M, et al. Analysis of epitope-specific immune responses induced by vaccination with structurally folded and unfolded recombinant Bet v 1 allergen derivatives in man. J Immunol 2007; 179:5309-16; PMID:17911617.
47. 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; 122:951-60; PMID:19000581; http://dx.doi.org/10.1016/j.jaci.2008.09.017.
48. Winther L, Poulsen L, Robin B, Melac 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; http://dx.doi.org/10.1016/j.jaci.2008.12.822.
49. Valenta R, Linhart B, Swoboda I, Niederberger V. Recombinant allergens for allergen-specific immunotherapy: 10 years anniversary of immunotherapy with recombinant allergens. Allergy 2011; 66:775-83; PMID:21352238; http://dx.doi.org/10.1111/j.1398-9995.2011.02565.x.
50. Larché M. Peptide and recombinant immunotherapy. [xi.]. Immunol Allergy Clin North Am 2011; 31:377-89, xi; PMID:21530826; http://dx.doi.org/10.1016/j.iac.2011.03.008.
51. Moldaver D, Larché M. Immunotherapy with peptides. Allergy 2011; 66:784-91; PMID:21507007; http://dx.doi.org/10.1111/j.1398-9995.2011.02610.x.
52. Kündig TM, Senti G, Schnetzler G, Wolf C, Prinz Vavricka BM, Fulurija A, et al. Der p 1 peptide on virus-like particles is safe and highly immunogenic in healthy adults. J Allergy Clin Immunol 2006; 117:1470-6; PMID:16751015; http://dx.doi.org/10.1016/j.jaci.2006.01.040.
53. Larché M. Peptide immunotherapy for allergic diseases. Allergy 2007; 62:325-31; PMID:17298351; http://dx.doi.org/10.1111/j.1398-9995.2006.01309.x.
54. 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; 8:1937-45; PMID:8982778; http://dx.doi.org/10.1093/intimm/8.12.1937.
55. 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; 154:1623-8; PMID:8970345.
56. Maguire P, Nicodemus C, Robinson D, Aaronson D, Umetsu DT. The safety and efficacy of ALLERVAX CAT in cat allergic patients. Clin Immunol 1999; 93:222-31; PMID:10600332; http://dx.doi.org/10.1006/clim.1999.4795.
57. Haselden BM, Kay AB, Larché M. Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. J Exp Med 1999; 189:1885-94; PMID:10377184; http://dx.doi.org/10.1084/jem.189.12.1885.
58. Haselden BM, Larché 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; 108:394-401; PMID:11544459; http://dx.doi.org/10.1067/mai.2001.117460.
59. Oldfield WL, Kay AB, Larché 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; 167:1734-9; PMID:11466398.
60. Oldfield WL, Larché M, Kay AB. Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. Lancet 2002; 360:47-53; PMID:12114041; http://dx.doi.org/10.1016/S0140-6736(02)09332-7.
61. Alexander C, Ying S, B Kay A, Larché M. Fel d 1-derived T cell peptide therapy induces recruitment of CD4+ CD25+; CD4+ interferon-gamma+ T helper type 1 cells to sites of allergen-induced late-phase skin reactions in cat-allergic subjects. Clin Exp Allergy 2005; 35:52-8; PMID:15649266; http://dx.doi.org/10.1111/j.1365-2222.2005.02143.x.
62. Alexander C, Tarzi M, Larché M, Kay AB. The effect of Fel d 1-derived T-cell peptides on upper and lower airway outcome measurements in cat-allergic subjects. Allergy 2005; 60:1269-74; PMID:16134993; http://dx.doi.org/10.1111/j.1398-9995.2005.00885.x.
63. Müller 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; 101:747-54; PMID:9648701; http://dx.doi.org/10.1016/S0091-6749(98)70402-6.
64. 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; 111:854-61; PMID:12704369; http://dx.doi.org/10.1067/mai.2003.1337.
65. Tarzi M, Klunker S, Texier C, Verhoef A, Stapel SO, Akdis CA, et al. Induction of interleukin-10 and suppressor of cytokine signalling-3 gene expression following peptide immunotherapy. Clin Exp Allergy 2006; 36:465-74; PMID:16630151; http://dx.doi.org/10.1111/j.1365-2222.2006.02469.x.
66. Crameri R, Kündig TM, Akdis CA. Modular antigen-translocation as a novel vaccine strategy for allergen-specific immunotherapy. Curr Opin Allergy Clin Immunol 2009; 9:568-73; PMID:19680120; http://dx.doi.org/10.1097/ACI.0b013e3283310fdf.
67. Martínez-Gómez 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; 64:172-8; PMID:19076537; http://dx.doi.org/10.1111/j.1398-9995.2008.01812.x.
68. Zaleska A, Soyer O, Eiwegger T, Bassin C, Sollner S, Palomares O, et al. Immune regulation by intralymphatic immunotherapy with modular allergen-translocation MAT vaccine. Allergy 2011; 66:1-104.
69. 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; 34:1525-33; PMID:15479266; http://dx.doi.org/10.1111/j.1365-2222.2004.02081.x.
70. Saarne T, Neimert-Andersson T, Grönlund H, Jutel M, Gafvelin G, van Hage M. Treatment with a Fel d 1 hypoallergen reduces allergic responses in a mouse model for cat allergy. Allergy 2011; 66:255-63; PMID:20804464; http://dx.doi.org/10.1111/j.1398-9995.2010.02468.x.
71. Niespodziana K, Focke-Tejkl M, Linhart B, Civaj V, Blatt K, Valent P, et al. A hypoallergenic cat vaccine based on Fel d 1-derived peptides fused to hepatitis B PreS. J Allergy Clin Immunol 2011; 127:1562-70 e6.
72. Valenta R, Niespodziana K, Focke-Tejkl M, Marth K, Huber H, Neubauer A, et al. Recombinant allergens: what does the future hold? J Allergy Clin Immunol 2011; 127:860-4; PMID:21458656; http://dx.doi.org/10.1016/j.jaci.2011.02.016.