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Peptide-Based Allergen Specific Immunotherapy for the Treatment of Allergic Disorders

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Abstract: Allergen specific immunotherapy (ASIT) and environmental control are the only etiologic treatments of allergic rhino-conjunctivitis, asthma and atopic dermatitis. The clinical benefit of ASIT relies on the selection of the patients and the identification and administration of the allergen, or allergens. Different routes of administration have been investigated, including subcutaneous, intradermal, epicutaneous, sublingual, inhaled, or intra-lymphatic. While subcutaneous and sublingual allergen specific immunotherapy may require from 3 to 5 years of treatment, clinical efficacy with intra-lymphatic treatment can be achieved after 3 injections. The most severe side effect of ASIT is anaphylaxis. Novel approaches are being investigated to reduce the allergenicity of immunotherapy vaccines, maintaining immunogenicity. Peptide immunotherapy has been directed mostly against autoimmune diseases, but the use of synthetic peptides for ASIT is a promising field in basic science, applied immunology and in clinical development. Short synthetic peptides bear allergen-specific CD4 T-cell epitopes which induce tolerance by stimulating regulatory (Treg) and Th1 cells. In the present patent review, we describe new trends in allergen immunotherapy using peptides, which, from a clinical point of view, are promising.

Keywords: Allergen peptides, allergen specific immunotherapy, *Blomia tropicalis*, *Dermatophagoides pteronyssinus*, subcutaneous, sublingual, intradermic, intralymphatic.

INTRODUCTION

Allergen specific immunotherapy (ASIT) and environmental control are considered as the only etiologic treatments of allergic diseases, such as allergic rhino-conjunctivitis, asthma and atopic dermatitis. It is firmly established that ASIT can alter the natural course of the disease [1, 2]. The administration of increasing doses of an allergen extract by subcutaneous, sublingual, intradermic, or intradermal routes, modulate immune response of the allergic individual It has been demonstrated that ASIT modifies disease course and consequently is clinically efficient. ASIT interferes with basic immunological mechanisms related to the disease, and it induces tolerance. Treatment results in persistent reduction, disappearance, or even cure of the allergic symptoms. The clinical benefits of ASIT rely on the selection of the patients and the identification and administration of the allergens. While the traditional route of administration is subcutaneous (SCIT), in the recent years, other routes have been investigated, such as sublingual, inhaled, intra-dermal, epicutaneous or intra-lymphatic.

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Currently, only non-modified, or aldehyde modified allergen preparations are available in the market. The use of non-modified allergen extracts is associated with an increased amount of adverse reactions during treatment, especially during the build-up Phases [3, 4]. The most severe side effect is anaphylaxis [5]. Thus, this treatment requires precaution from patients, attending doctors, and medical personnel. Novel approaches are being investigated to reduce the allergenicity of immunotherapy vaccines while retaining the immunogenicity of the allergen molecules.

The recommended duration of ASIT is usually from 3 to 5 years for sublingual and subcutaneous treatments, whereas for intra-lymphatic treatments, efficacy can be reached after 3 injections, although long term efficacy studies are not currently available. In general, the treatment course is divided into two Phases: the step up or increase dose Phase and the maintenance Phase. The recommended maintenance it involves injections every 4 weeks. After each injection, the patient has to remain under medical surveillance for 30 minutes due to the risk of anaphylactic reactions even though are extremely rare. The clinic center should be equipped to support emergency treatment [6]. ASIT is dose dependent [7], however, the allergen dose depends upon the appearance systemic reactions.

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One approach that is gaining considerable attention and support is the use of short synthetic peptides bearing allergen-specific CD4 T-cell epitopes which induce tolerance by stimulating regulatory (Treg) and Th1 cells [8, 9]. Short peptides exhibit a significantly reduced capacity to crosslink immunoglobulin (Ig)E and consequently do not activate mast cells and basophils due to the lack of tertiary structure [10]. Peptides and anti-peptide antibodies are used in biochemistry and molecular biology for the purification and characterization of specific oligopeptides and proteins, as well as for the identification and mapping of antibody binding sites. Antigenic determinants are classified into linear and non-linear. T cell epitopes correspond to linear antigen peptide fragments, that directly bind to major histocompatibility molecules (MHC) displayed in the cell surface of antigen presenting cells (APC), Non-linear epitopes are composed of non-contiguous residues which are not adjacent in the protein primary sequence but become contiguous by a three-dimensional folding or epitope configuration. Short amino acid fragments derived from the parent protein antigen may induce, or increase, immune response. There are several studies that have shown that short T cell epitope-based peptides of major allergens are sufficient to effectively treat cat [11, 12] and bee venom [13, 14] allergies.

Progress has been reported in cat immunotherapy using peptides. A newer version of a PIT vaccine (Cat-PAD) with a reduced number of peptides (from 27 in early studies to 7) and thioglycerol to prevent cross-linking of IgE has been developed [15]. In a Phase IIa clinical trial, a single intradermal dose was well tolerated. A follow up study, evaluated 2 regimens of Cat-PAD peptide immunotherapy in 202 patients with cat allergy [16]. Patients received 6 nanomolar Cat-PAD injections every 4 weeks (total of 4 injections). Afterwards, and for 4 successive days, they were placed in an exposure chamber to determine whether the active treatment improved allergic rhino-conjunctivitis symptoms. They found beneficial effects in the first year of treatment. Patients who received active treatment experienced persistent reduction in symptoms 1 year after the 4 injections [16]. This data suggests that synthetic peptides may offer an easier immunotherapy strategy without requiring dose escalation and that the clinical benefits may last for up to 1 year.

Recent patents to improve allergen immunotherapy vaccines have been published by our group in this issue [17].

1. PIT WITH HOUSE DUST MITE PEPTIDES

A. Fusion Proteins with Representation of Different Allergens: Vaccine Proposal for Mite Allergies (EP2727934)

The inhalation of house dust mites (HDM) is one of the most important risk factors associated with the development of allergic rhinitis, asthma and rhino-conjunctivitis worldwide [18]. More than 50% of all allergic patients suffer from HDM-allergy. The species that are most often implicated in allergic reactions belong to the genus *Dermatophagoides*, namely *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*. Their optimal growth conditions include temperatures of about 25ºC and relative humidities above 70%. In tropical and sub-tropical regions, *Blomia tropicalis*is also very common, being, in some cases, the predominant mite species in house dust. In these regions, individuals allergic to mites are mostly sensitized to *D. pteronyssinus* and *B. tropicalis* [19].

The patent EP2727934 reports a vector referred as pET45b/PF3a constituted by 5852 base pairs and a vector referred as pET45b/PF14c constituted by 5719 base pairs, which, when introduced into *Escherichia coli*, allows the expression of recombinant proteins PF3a having 196 amino acids and PF14c having 152 amino acids [20]. PF3a comprises different fragments of allergens from the mite species *B. tropicalis* and *D. pteronyssinus*; Blo t 5, Der p 2, Blo t 10, Der p 8, Blo t 8, Der p 1, Der p 2 and Der p 7.

PF14c corresponds to different fragments of 5 allergens from mite *B. tropicalis*, Blo t 5, Blo t 8, Blo t 10, Blo t 12 y Blo t 13 [20]. According to the present invention, this potential vaccine to treat mite allergy is characterized by an amino acid sequence selected from the group consisting of PF3a and PF14c or a mix thereof [20]. Authors have shown that these polypeptides have low allergenicity but more studies are needed to evaluate if this vaccine is useful.

B. Peptide for Vaccine Design (US20140186403)

The authors of the present invention have discovered that the mix of peptide fragments from the mite allergens Der p 1, Der f 1, Der p 2, Der f 2, Der p 7 and Der f 7 in allergy vaccines are useful in treating allergic individuals [21]. The peptides have been selected to retain T cell specificity whilst being small enough in size do not possess significant tertiary structure that would enable them to retain the conformation of an IgE-binding epitope. Authors have produced peptide combinations with the following features:

- The combined peptides bind strongly to the seven most common human MHC Class II HLA-DRB1 allotypes selected by EpiMatrix analysis.
- The peptide combination gives significant stimulation of cytokine release (IFN-γ and IL-13) in HDM allergic individuals.

More clinical trials are needed to assess efficacy and safety for threating HDM allergic patients with this PIT.

2. HYPOALLERGENIC VARIANTS OF THE MAJOR ALLERGEN FROM *BETULA VERRUCOSA* POLLEN (EP2201031)

Bet v 2 is a 14 kDa allergic protein from *Betula verrucosa*. Bet v 2 belongs to the profilin family, which are ubiquitous cytoplasmic proteins involved in the regulation of the cytoskeleton of eukaryotic cells. Profilins have been identified as allergens in the pollen from trees, grasses and weeds and in many fruits and vegetables. It is therefore defined as a panallergen, despite the fact that they are only allergenic

(bind specific IgE) in 20% of the patients allergic to pollen [22]. The high sequence homology, greater than 60% in most plant profilins of various origins, causes cross-sensitization not only with pollen from botanically related and unrelated plant species, but also between pollen and vegetable foods [23] or between pollen and latex [24]. Many studies have confirmed the immunologic equivalence of profilins. It has been shown that specific IgE from patients sensitized to a specific profilin are able to cross react to profilins of different origins. The IgE binding to different profilins can be mutually inhibited [23]. The high degree cross-reactivity between different profilins allows the use of a single profilin for allergy diagnosis. Recombinant Bet v 2 is often used as the allergen of choice for profilin-specific IgE determinations [25]. The authors of this potential vaccine provide information about hypoallergenic sequence variants of Bet v 2, nucleic acid encoding them, pharmaceutical compositions containing the same and their use in the prophylaxis and therapy of allergic diseases caused by pollen of the birch tree *B. verrucosa* [26]. Authors have replaced one or more amino acid residues within the sequence of Bet v 2 rendering it less reactive to IgE antibodies. The variants obtained by substitution of one, or more residues are identified as SEQ ID NO:2 (1 residue substitution), SEQ ID NO:3 (1 res. subst.), SEQ ID NO:4 (2 res. subst.), SEQ ID NO:5 (3 res. subst.) and SEQ ID NO:6 (3 res. subst.). The IgE reactivity of the proteins SEQ ID NOs: 2-6 was tested in an ELISA assay with a pool of sera of allergic patients. As compared to the wild-type (wt) a mean reduction of IgE reactivity was observed for Bet v 2 allergen (SEQ ID NO: 1), a 92% (SEQ ID NO: 2), 13% (SEQ ID NO:3), 97% (SEQ ID NO:4) and 93% (SEQ ID NO.s:5 and 6). These results were confirmed by RAST inhibition.

In experiments performed with Balb/c mice, the Bet v, the 2 wild-type allergen, and the hypoallergenic protein SEQ ID NO: 5 were able to induce an IgG-specific immune response [26].

This invention provides an interesting vaccine for use in profilin allergic patients who now only have the possibility of avoidance measures.

3. HYPOALLERGENIC MUTANT POLYPEPTIDES BASED ON FISH PARVALBUMIN (US20140121356)

Milk, egg, peanuts, tree nuts, shellfish and fish represent the most important sources of allergens in IgE mediated food hypersensitivity [27]. Parvalbumins, small calcium-binding proteins, show a remarkable resistance to heat, denaturing chemicals and proteolytic enzymes. These proteins are present in high quantities in the white muscle of lower vertebrates and in lower amounts in fast twitch muscles of high vertebrates. Parvalbumins have been identified as major fish allergens and responsible of cross-reactivity [28, 29]. These proteins belong to the EF-hand superfamily of calcium-binding proteins. Parvalbumins contain three EF-hand [30], AB, CD and EF sites. CD and EF sites are paired to form a stable domain capable of binding two cations Ca^{2+} or Mg^{2+}. However, AB is unable to bind cations and acts as a stabilizing element [31].

The authors of the present invention produced the first recombinant fish parvalbumin (rCyp c 1.01) with immunological features similar to the natural allergen [32]. rCyp 1.01 reacted with IgE from all patients allergic to fish tested. It induced specific and dose-dependent basophil histamine release and it contained most of the specific IgE binding epitopes 70%) present in natural allergen extracts from various fish species. The authors of the patent US20100216717 propose that one possibility to obtain hypoallergenic parvalbumin derivatives is by using site-directed mutagenesis of critical amino acids either within, or outside of IgE binding epitopes. This manipulation alters the fold of the molecule and decreases the secondary structure content of the protein [33]. The calcium-binding domains were also mutated by replacing the first and third amino acid of the calcium binding loops (Asp) by non-polar Ala residues [34]. The resulting proteins were termed Mut-CD (mutation in CD domain), Mut-EF (mutation in EF domain) and Mut-CD/EF (mutation in both domains). Mutation in Mut-CD/EF caused a significant change of conformational epitope and/or unfolding of the protein. Thus, IgE binding was completely abolished in the sera all of the allergic patients tested. This invention is interesting and promising, since hypoallergenic parvalbumin molecules will therefore open up new paths for the immunotherapy of IgE-mediated fish allergies.

4. GRASS PEPTIDES FOR VACCINES (US8753644)

Authors describe the development of a vaccine based on recombinant hypoallergenic hybrid molecules named A-Q, and constructed by the assembly of allergen fragments derived from 4 major timothy (*Phleum pratense*) grass pollen allergens Phl p 1, Phl p 2, Phl p 5, and Phl p 6 for the treatment of grass pollen allergy [35]. Codon-optimized synthetic genes encoding building blocks and combinations of the 4 allergens were designed according to epitope mapping studies and structural data and subsequently expressed in *Escherichia coli*. Seventeen recombinant hybrid molecules were purified by affinity chromatography and evaluated regarding expression, purity and fold, solubility, and reduced allergenic activity. Four hypoallergenic hybrid molecules (B, C, P and Q), were identified with low allergenicity and maintained immunogenicity.

Other authors [36] produced peptide combinations derived from allergens Phl p 1 y Phl p 5, with the following characteristics:

- The combination binds to many different MHC Class II molecules (HLA-DR) selected by EpiMatrix analysis.
- The peptide combination gives significant stimulation of cytokine release (IFN-γ, IL-10 and IL-13) in 48 grass allergic individuals.

- The seven peptide combinations do not produce significant histamine release in any of 42 assays tested.

These hypoallergenic hybrid molecules represent safe vaccines for immunotherapy of grass pollen allergy but more clinical trials are clearly needed.

5. PEANUT ALLERGY IMMUNOTHERAPY (WO2014066939)

Peanut allergy is the most frequent cause of food-induced anaphylaxis world-wide [37]. Avoidance is currently the only therapeutic option. Of the 11 peanut allergens currently identified, Ara h 1 and Ara h 2 are considered major allergens due to a high frequency of specific IgE binding [38, 39]. Ara h 1 is the most abundant major allergen in peanut making up from 12% to 16% of the total peanut protein [40]. It is a 7S seed storage glycoprotein, or vicilin, and the expression of the protein is associated with peanut maturity [41]. Prickett *et al.* have identified short CD4+ T cell epitope-based Ara h 1 peptides that target allergen-specific T cells without increasing allergenicity. The authors reported 10 T cell epitopes of Ara h 1 incorporated into a panel of 7 short peptides. The authors have also identified 3 T cell epitopes of Ara h 2 [42]. The combinations of all these T cell epitopes from Ara h 1 and Ara h 2 could provide broad acting and safe peptide immunotherapy for peanut allergy. The same authors also identified selected sequences of core T cell epitope regions which are immune-dominant in other Ara h 1 peptide fragments, and presented by HLA-DQ molecules [43]. Ara h 1-specific oligoclonal T cell lines were generated from peripheral blood mononuclear cells (PMBC) of peanut allergic subjects [44] as described [42] with crude peanut extract (100pg/mL), Ara h 1 (10gg/mL) or 20-mer peptides spanning the Ara h 1 sequence (11 amino acid) overlap (17 amino acid overlap for the last peptide). A total of 145 Ara h 1-specific T cell lines were generated. All of them recognized epitopes throughout the Ara h 1 sequence. Basophil reactivity to peptides was assessed in sera from seven peanut allergic patients. None of the candidate peptides induced cell activation at any concentration tested.

More clinical trials with these peptides are needed to assess the safety and effectiveness when used for PIT. This vaccine, if successful, may change the quality of life of thousands of peanut allergic patients, worldwide.

6. BEE VENOM HYPERSENSITIVITY

A previous clinical study, which treated 5 bee venom-allergic subjects with immunodominant T cell epitopes of Api m 1, showed that a wild bee sting was only tolerated by three subjects, with the remaining two developing mild systemic allergic reactions [45]. Immunotherapy with peptides containing T cell epitopes of the major bee venom allergen phospholipase A2 (Api m 1) provides a safe and effective treatment without important adverse events [13]. All five patients, after two months of subcutaneous immunotherapy treatment, tolerated challenges with phospholipase A2 first, and bee sting one week later. Prophylactic administration of peptides from the bee venom allergen Api m 4 (melitin), or the hornet venom allergen Dol m 5 to mice sensitized to whole venom allergens resulted in a partial reduction in both T cell proliferation and B cell antibody responses to subsequent allergen sensitization [46]. In 2003, another study with three synthetic polypeptides covering the whole Api m 1 molecule, authors observed an increase of interferon-γ and IL-10 but not Th2 cytokines [47].

7. JAPANESE CEDER POLLINOSIS

Japanese cedar (*Cryptomeria japonica*) pollinosis is a serious type I allergic disease in Japan [48]. More than 25% of Japanese citizens suffer from *C. japonica* pollinosis and the number of patients has constantly increased over the last years [49]. Sone *et al.* designed a recombinant polypeptide containing the major five T cell epitopes (aa 106-120 and aa 211-225 from Cry j 1 and aa 66-80, aa 182-200, and aa 346-360 from Cry j 2)[50]. Hirahara *et al.* [51] prepared a hybrid peptide comprising seven T cell epitopes (aa 212-224, aa 235-247 and aa 312-330 from Cry j 1 and aa 77-89, aa 96-107, aa 192-204, and aa 356-367 from Cry j 2). Both polypeptides are good candidates for immunotherapy against Japanese cedar pollinosis. Takagi *et al.* [52] studied the efficacy of transgenic rice seeds expressing Cry j 1 and Cry j 2 T cell epitope peptides for the induction of systemic unresponsiveness to pollen allergens of Japanese cedar in 2005. A group of mice was fed with the transgenic rice seeds and two other groups of mice received non-transgenic rice seeds or phosphate-buffered saline. In all of them the treatment was effective in the suppression of allergen-specific IgE responses. It further inhibits histamine release by blocking the formation of the allergen-IgE complex. Kawabe *et al.* [53] have recently developed a treatment for pollen allergy by inducing oral tolerance using manipulated chickens by retroviral gene transduction, to produce a fusion protein of chicken egg white lysozyme and a peptide derived from 7 dominant human T-cell epitopes of Japanese cedar pollen allergens (cLys-7crp). A murine model of *C. japonica* pollinosis was developed to evaluate the therapeutic efficacy of oral administration of cLys-7crp produced in the egg white of a genetically manipulated hen. The authors indicated that the maximum single dose was limited by the volume (0.3ml) of egg white injectable to a mouse using the feeding needle. Further studies are needed to assess the use of eggs containing the therapeutic proteins produced by GM chickens as a therapeutic food.

Recently, an immune-regulatory liposome encapsulating the recombinant fusion protein of Cry j 1-Cry j 2 was manufactured as a novel vaccine for Japanese cedar polli-

nosis without risk of anaphylaxis [54]. In a mouse model sensitized with native Cry j 1 and Cry j 2, the vaccine which incorporated the Cry j 1-Cry j 2 fusion proteins in the immune-regulatory liposome showed suppression of IgE and IgG antibody responses after being challenged with the allergens. Masuyama *et al*. using these T-cell lines, 37 Cry j 1-derived overlapping peptides were assessed for their proliferative responses and cytokine production. Four peptides corresponding to the Cry j 1 sequence were able to induce proliferative responses to more than one T-cell line. The authors suggested that 4 Cry j 1-derived peptides (p61-80, p115-132, p206-225 and p337-353) may be considered to be the immune-dominant T-cell epitopes of the Cry j 1 molecule, and can be useful for the design of peptide-based immunotherapy for the management of Japanese cedar pollinosis [55].

The hybrid fusion allergen is expected to provide safer and more effective vaccines for immunotherapy. Vaccines using only T cell epitopes are also safer than native allergens, but there is wide variation among individual T cell epitopes. The fusion protein of major allergens covers all sequential T cell epitopes but is expected to have less IgE-binding capacity because its three dimensional structure is disrupted in some B cell epitopes.

We have summarized the patents of the article in the Table **1**.

CURRENT & FUTURE DEVELOPMENTS

The development of novel strategies to overcome the potential side effects associated with allergen immunotherapy is very important. It depends to a large degree on a deep and accurate understanding of the immunological basis and mechanisms of the disease and of its successful treatment using ASIT. The development of high-throughput technologies and the wide use of bioinformatics, proteomic and peptidomic research offer new opportunities to analyze the interaction of peptides with the immune system. Peptide associated antigenicity, immunogenicity and pathogenicity are also being investigated [56]. It is necessary to establish uniform criteria for the positive identification of immune-reactive peptides in order to design effective peptide-immunotherapies. Thus, it is important to define whether the repertoire of allergen specific CD4 T cell epitopes recognized during allergy remains the same one that induces tolerance. Allergen specific PIT has found been successfully treated allergic patients, and there are many opportunities for the application of PIT in this category. The advances in immunology and bioengineering are being applied to biologists to improve their clinical efficacy and feasibility of production by optimizing their design and clinical efficacy [57]. The fields of Allergy and Immunology are pioneer in several of these applications and may experience an expanding use of PIT in this field.

Table 1. Summary of the Patents Included in the Present Article.

Patent	Content
Fusion proteins with representation of different allergens: vaccine proposal for mite allergies (EP2727934)	Recombinant DNA molecules codifying fused peptides from different allergens from *Blomia tropicalis*and *Dermatophagoides pteronyssinus*
Peptide for vaccine (US20140186403)	Combinations of peptide fragments from Group 1 (Der p 1, Der f 1), Group 2 (Der p 2, Der f 2) and Group 3 (Der p 7, Der f7) mite allergens
Hypoallergenic variants of the major allergen from *Betula verrucosa* pollen (EP2201031)	Hypoallergenic sequence variants of the Bet v 2 protein
Hypoallergenic mutant polypeptides based on fish parvalbumin (US20140121356)	Non-naturally occurring polypeptides derived from fish allergens such as parvalbumin Cyp c 1.01 from carp
Grass peptides for vaccines (US8753644)	Peptide combinations from Timothy, Perennial Rye and Bermuda grasses target multiple different MHC class II molecules and stimulate cytokine release
Peanut allergy immunotherapy (WO2014066939)	Peptides immune-reactive with T cells in subjects having allergy to the Ara h 1 allergen
Bee venom hypersensitivity	Immunotherapy with peptides containing T cell epitopes
Japanese ceder pollinosis	Peptide-based immunotherapy of Japanese cedar pollinosis

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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Declared none.

REFERENCES

[1] Viswanathan RK, Busse WW. Allergen immunotherapy in allergic respiratory diseases: From mechanisms to meta-analyses. Chest 2012; 141(5):1303-14.

[2] Abramson MJ, Puy RM, Weiner JM. Injection allergen immunotherapy for asthma. Cochrane Database Syst Rev 2010; 8: CD001186.

[3] Marfo K, Lu A, Ling M, Akalin E. Desensitization protocols and their outcome. Clin J Am Soc Nephrol 2011; 6(4): 922-36.

[4] Kannan JA, Epstein TG. Immunotherapy safety: What have we learned from surveillance surveys? Curr Allergy Asthma Rep 2013; 13(4): 381-8.

[5] Makatsori M, Calderon MA. Anaphylaxis: Still a ghost behind allergen immunotherapy. Curr Opin Allergy Clin Immunol 2014; 14(4): 316-22.

[6] Wallace DV. Anaphylaxis in the allergist's office: Preparing your office and staff for medical emergencies. Allergy Asthma Proc 2013; 34(2): 120-31.

[7] Frew AJ, Powell RJ, Corrigan CJ, Durham SR. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006; 117(2): 319-25.

[8] Mascarell L, Van Overtvelt L, Lombardi V, Razafindratsita A, Moussu H, Horiot S, *et al*. A synthetic triacylated pseudo-dipeptide molecule promotes Th1/TReg immune responses and enhances tolerance induction via the sublingual route. Vaccine 2007; 26(1):108-18.

[9] Reche PA, Fernandez-Caldas E, Flower DR, Fridkis-Hareli M, Hoshino Y. Peptide-based immunotherapeutics and vaccines. J Immunol Res 2014; 2014: 256784.

[10] Larche M. Immunotherapy with allergen peptides. Allergy Asthma Clin Immunol 2007; 3(2): 53-9.

[11] Alexander C, Ying S, B Kay Y, Larche 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(1): 52-8.

[12] Alexander C, Tarzi M, Larche 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(10):1269-74.

[13] 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; 101(6 Pt 1): 747-54.

[14] 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; 100(1): 96-103.

[15] Worm M, Lee HH, Kleine-Tebbe J, Hafner RP, Laidler P, Healey D, *et al*. Development and preliminary clinical evaluation of a peptide immunotherapy vaccine for cat allergy. J Allergy Clin Immunol 2011; 127(1): 89-97.

[16] Patel D, Couroux P, Hickey P, Salapatek AM, Laidler P, Larche M, *et al*. Fel d 1-derived peptide antigen desensitization shows a persistent treatment effect 1 year after the start of dosing: A randomized, placebo-controlled study. J Allergy Clin Immunol 2013; 131(1): 103-9 e1-7.

[17] El-Qutob D, Mencia G, Fernandez-Caldas E. Recent advances in immunotherapy for allergic diseases. Recent Pat Inflamm Allergy Drug Discov 2014; 8(1): 24-35.

[18] Platts-Mills TA, Chapman MD. Dust mites: Immunology, allergic disease, and environmental control. J Allergy Clin Immunol 1987; 80(6): 755-75.

[19] Jimenez S, Puerta L, Mendoza D, Caraballo L, Chua K. IgE antibody responses to recombinant allergens of *Blomia tropicalis*and *Dermatophagoides pteronyssinus* in a tropical environment. Allergy Clin Immunol Int 2007; 19(6): 233-8.

[20] Puerta, L.L., Caraballo, L., Cantillo, J.F. Fusion proteins with representation of different allergens: Vaccine proposal for mite allergies. EP2727934 (**2014**).

[21] Hafner, R.P., Laidler, P., Larche, M. Peptide for vaccine. US20140186403 (**2014**).

[22] Valenta R, Duchene M, Ebner C, Valent P, Sillaber C, Deviller P, *et al*. Profilins constitute a novel family of functional plant pan-allergens. J Exp Med 1992; 175(2): 377-85.

[23] van Ree R, Voitenko V, van Leeuwen WA, Aalberse RC. Profilin is a cross-reactive allergen in pollen and vegetable foods. Int Arch Allergy Immunol 1992; 98(2): 97-104.

[24] Ganglberger E, Radauer C, Wagner S, Riordain G, Beezhold DH, Brehler R, *et al*. Hev b 8, the *Hevea brasiliensis* latex profilin, is a cross-reactive allergen of latex, plant foods and pollen. Int Arch Allergy Immunol 2001; 125(3): 216-27.

[25] Fedorov AA, Ball T, Mahoney NM, Valenta R, Almo SC. The molecular basis for allergen cross-reactivity: Crystal structure and IgE-epitope mapping of birch pollen profilin. Structure 1997; 5(1): 33-45.

[26] Mistrello, G., Zanotta, S., Roncarolo, D., Falagiani, P. Hypoallergenic variants of the major allergen from *Betula verrucosa* pollen. EP2201031 (**2014**).

[27] Bischoff SC, Herrmann A, Manns MP. Prevalence of adverse reactions to food in patients with gastrointestinal disease. Allergy 1996; 51(11): 811-8.

[28] Lehky P, Blum HE, Stein EA, Fischer EH. Isolation and characterization of parvalbumins from the skeletal muscle of higher vertebrates. J Biol Chem 1974; 249(13): 4332-4.

[29] Pechere JF. The significance of parvalbumin among muscular calcium proteins, in Calcium-Binding Proteins and Calcium, Wasserman R, Corradino R, Carafoli R, Kretsinger D, MacLennan D, Siegel F, Editors, Elsevier: Holland 1997; 213.

[30] Ikura M. Calcium binding and conformational response in EF-hand proteins. Trends Biochem Sci 1996; 21(1):14-7.

[31] Declercq JP, Tinant B, Parello J, Rambaud J. Ionic interactions with parvalbumins. Crystal structure determination of pike 4.10 parvalbumin in four different ionic environments. J Mol Biol 1991; 220(4):1017-39.

[32] Swoboda I, Bugajska-Schretter A, Valenta R, Spitzauer S. Recombinant fish parvalbumins: Candidates for diagnosis and treatment of fish allergy. Allergy 2002; 57 (Suppl 72): 94-6.

[33] Valenta, R., Valent, P., Spitzauer, S., Swoboda, I. Hypoallergenic mutant polypeptides based on fish parvalbumin. US20100216717 (**2010**).

[34] Valenta, R., Valent, P., Spitzauer, S., Swoboda, I. Hypoallergenic mutant polypeptides based on fish parvalbumin. US20140121356 (**2014**).

[35] Valenta, R., Linhart, B., Focke-Tejkl, M., Neubauer, A., Valent, P., Blatt, K. Hypoallergenic hybrid polypeptides for the treatment of allergy. EP2475386 (**2014**).

[36] Hafner, R.P., Laidler, P., Layton, G., Larche, M. Grass peptides for vaccine. US8753644 (**2014**).

[37] Bock SA, Munoz-Furlong A, Sampson HA. Further fatalities caused by anaphylactic reactions to food, 2001-2006. J Allergy Clin Immunol 2007; 119(4): 1016-8.

[38] de Leon MP, Rolland JM, O'Hehir RE. The peanut allergy epidemic: Allergen molecular characterisation and prospects for specific therapy. Expert Rev Mol Med 2007; 9(1): 1-18.

[39] Husain Z, Schwartz RA. Food allergy update: More than a peanut of a problem. Int J Dermatol 2013; 52(3): 286-94.

[40] Koppelman SJ, Vlooswijk RA, Knippels LM, Hessing M, Knol EF, van Reijsen FC, *et al*. Quantification of major peanut allergens Ara h 1 and Ara h 2 in the peanut varieties Runner, Spanish, Virginia, and Valencia, bred in different parts of the world. Allergy 2001; 56(2):132-7.

[41] Pomes A, Butts CL, Chapman MD. Quantification of Ara h 1 in peanuts: Why roasting makes a difference. Clin Exp Allergy 2006; 36(6): 824-30.

[42] Prickett SR, Voskamp AL, Dacumos-Hill A, Symons K, Rolland JM, O'Hehir RE. Ara h 2 peptides containing dominant CD4+ T-cell epitopes: Candidates for a peanut allergy therapeutic. J Allergy Clin Immunol 2011; 127(3): 608-15 e1-5.

[43] O'Hehir, R., Rolland, J., Prickett, S. Novel immunotherapeutic molecules and uses thereof. WO2014066939 (**2014**).

[44] Mannering SI, Dromey JA, Morris JS, Thearle DJ, Jensen KP, Harrison LC. An efficient method for cloning human autoantigen-specific T cells. J Immunol Methods 2005; 298(1-2): 83-92.

[45] Carballido JM, Carballido-Perrig N, Kagi MK, Meloen RH, Wuthrich B, Heusser CH, *et al*. T cell epitope specificity in human allergic and nonallergic subjects to bee venom phospholipase A2. J Immunol 1993; 150(8 Pt 1): 3582-91.

[46] King TP, Lu G, Agosto H. Antibody responses to bee melittin (Api m 4) and hornet antigen 5 (Dol m 5) in mice treated with the dominant T-cell epitope peptides. J Allergy Clin Immunol 1998; 101(3): 397-403.

[47] 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(4): 854-61.

[48] Okuda M. Epidemiology of Japanese cedar pollinosis throughout Japan. Ann Allergy Asthma Immunol 2003; 91(3) :288-96.

[49] Okamoto Y, Horiguchi S, Yamamoto H, Yonekura S, Hanazawa T. Present situation of cedar pollinosis in Japan and its immune responses. Allergol Int 2009; 58(2):155-62.

[50] Sone T, Morikubo K, Miyahara M, Komiyama N, Shimizu K, Tsunoo H, *et al*. T cell epitopes in Japanese cedar (*Cryptomeria japonica*) pollen allergens: Choice of major T cell epitopes in Cry j 1 and Cry j 2 toward design of the peptide-based immunotherapeutics for the management of Japanese Cedar pollinosis. J Immunol 1998; 161(1): 448-57.

[51] Hirahara K, Tatsuta T, Takatori T, Ohtsuki M, Kirinaka H, Kawaguchi J. *et al*. Preclinical evaluation of an immunotherapeutic peptide comprising 7 T-cell determinants of Cry j 1 and Cry j 2, the major Japanese cedar pollen allergens. J Allergy Clin Immunol 2001; 108(1): 94-100.

[52] Takagi H, Hiroi T, Yang L, Tada Y, Yuki Y, Takamura K, *et al*. A rice-based edible vaccine expressing multiple T cell epitopes induces oral tolerance for inhibition of Th2-mediated IgE responses. Proc Natl Acad Sci USA 2005; 102(48): 17525-30.

[53] Kawabe Y, Hayashida Y, Numata K, Harada S, Ito A, Kamihira M. Oral immunotherapy for pollen allergy using T-cell epitope-containing egg white derived from genetically manipulated chickens. PLoS One 2012; 7(10): e48512.

[54] Fujimura T, Okamoto Y. Antigen-specific immunotherapy against allergic rhinitis: The state of the art. Allergol Int 2010; 59(1): 21-31.

[55] Masuyama K, Chikamatsu K, Ikagawa S, Matsuoka T, Takahashi G, Yamamoto T, *et al*. Analysis of helper T cell responses to Cry j 1-derived peptides in patients with nasal allergy: Candidate for peptide-based immunotherapy of Japanese Cedar pollinosis. Allergol Int 2009; 58(1): 63-70.

[56] Sigalov, A. Multichain Immune Recognition Receptor Signaling: From Spatiotemporal Organization to Human Disease Springer Science & Business Media: 2008

[57] 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(3): 608-13.