

REVIEW ARTICLE

New Treatments for Allergy: Advances in Peptide Immunotherapy

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Abstract: Background: Nowadays, allergen-specific immunotherapy (AIT) is the only treatment able to modulate the course of allergic diseases. Although it has been applied for the last 100 years, treatment with whole allergen extracts is not without its drawbacks: AIT can cause local and systemic adverse events and may produce new IgE sensitization against other allergens present in the extract. Furthermore, the lengthy treatment duration (3-5 years), frequent administration, and high cost of treatment are other disadvantages. For these reasons, there is a need for safer and more effective AIT strategies. One promising approach is the use of synthetic peptides representing the B- or T-cell epitopes of allergens.

Objective: This review summarizes the main advances in peptide immunotherapy, from preclinical models to early clinical trials, focusing on house dust mite, bee venom, cat allergy, and *Oleaceae* pollinosis.

Results: Following an extensive review of the relevant literature, we summarize how peptide therapies may change the course of allergic diseases and promote allergen tolerance, thereby ameliorating the main disadvantages of AIT. Although the molecular mechanisms involved are not yet fully defined, they seem to depend on structure, length, peptide sequence, and route of administration. This novel immunotherapy has been demonstrated to modulate the immune system, promoting regulatory T-cell induction and Th2 inhibition. This tolerance-inducing potential has led this therapy to be termed SPIRE (synthetic peptide immuno-regulatory epitopes).

Conclusion: Experimental models and clinical trials have demonstrated the usefulness of SPIRE treatment to cure these diseases, opening a new era in allergen therapeutics.

Keywords: Allergy, immunoregulation, peptide immunotherapy, regulatory T cells, SPIRE, T-cell epitopes.

1. INTRODUCTION

1.1. Current Status of Allergen-Specific Immunotherapy (AIT)

1.1.1. Molecular Bases of AIT

The immune system (IS) requires tight control to protect the organism from exaggerated stimulatory signals. This control is characterized by functional inactivation mainly due to specific T-cell subtypes with an immunosuppressive function, generically named regulatory T-cells (Tregs). Depending on the nature of the

antigen, an imbalance in these regulatory mechanisms under certain environmental conditions can lead to allergic diseases or autoimmune disorders in genetically predisposed subjects [1].

Allergic diseases are highly complex adverse reactions of the IS against otherwise harmless substances. The pathophysiology of allergic diseases is complex and may be influenced by many factors, including genetic susceptibility and aspects of the microenvironment, such as allergen dose and route of exposure. In the allergic response, specific regions of antigens called epitopes are recognized by naïve T and B lymphocytes. First, allergens are recognized and presented to naïve T-cells by specific major histocompatibility complex (MHC) class-II antigens expressed on the surface of

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antigen-presenting cells (APC). T-cell activation induces the differentiation and expansion of T-helper type 2 (Th2) cells and leads to the production of interleukin (IL) 4, IL-5, and IL-13. These interleukins act on B cells, promoting immunoglobulin (Ig) class-switching to Ig type E (IgE). Allergen-specific IgE antibodies bind to high-affinity receptors for IgE (FcεRI) expressed on mast cells and basophils. Repeated exposure to the allergen causes the cross-linking of FcεRI-bound IgE, stimulating the release of histamine and other mediators responsible for the immediate symptoms of allergic disease. The late phase of the allergic reaction occurs 6-12 hours after allergen exposure, when allergen-specific cells are reactivated and expanded locally. Effector cells (*i.e.*, mast cells, basophils, and, specifically, eosinophils) release additional inflammatory mediators and cytokines, perpetuating the pro-inflammatory response. This phase is responsible for the symptoms of allergic diseases, and continuous exposure to the allergen causes disease chronicity [1, 2]. Fig. (1) summarizes the main phases of the allergic response.

Currently, allergic diseases constitute a global health problem. It is estimated that about 25% of the population is affected by type 1 reactions in industrialized countries [3, 4]. Although allergic symptoms can be often suppressed using various drugs, allergen-specific immunotherapy (AIT) is the only treatment that can modulate the course of allergic disease. AIT was first reported by Noon and Freeman in 1911 [5]. Their clinical trials proved that subcutaneous injection of grass pollen extract modulated the course of the disease, and more importantly, these effects remained more than a year after the conclusion of the treatment. The method used by the 2 researchers was quickly accepted by the scientific community, increasing this type of studies in subsequent years. Nowadays, AIT is a routine part of clinical practice in allergy [5].

AIT is the only treatment in allergy able to change the course of the disease. In contrast with other usual treatments as anti-inflammatory or bronchodilator drugs, AIT is not limited to control the symptoms of the disease. AIT is considered a therapeutic vaccine, and because it uses the patient IS to establish tolerance against specific antigens, its effects are long-term [2, 4]. However, although the molecular mechanisms involved in the AIT response have been extensively studied, they have not been elucidated in their entirety [6, 7]. AIT is believed to promote the absence of pro-inflammatory signals influenced by the maturation of dendritic cells (DC) into a tolerogenic response [2], thus inducing major changes in allergen-specific T-cell

subsets; this leads to a downregulation of the Th2 response with a shift towards the Th1 profile. T-cells with a regulatory phenotype are also promoted and their presence is associated with an increase in suppressive cytokines such as IL-10 and TGF-β [8, 9]. The effects on antibody responses are less clear. Further studies are needed to fully delineate their mechanisms of action. AIT often induces a transient increase in specific IgE synthesis, followed by a gradual decrease with continued treatment, although some meta-analyses have concluded that serum IgE levels remain stable in treated patients or the decrease occurs late, is relatively small, and is poorly correlated with clinical improvements [10]. It has been suggested that the production of antigen-specific IgG4 antibodies can antagonize and block the allergic response resulting from antigen recognition by IgE present on the surface of mast cells and basophils, which would limit activation and degranulation [11]. Therefore, the balance between IgE and IgG4 is crucial for successful AIT [10-12].

AIT also suppresses allergic inflammation induced by mast cells, basophils, and eosinophils. The recruitment of these cells to the site of allergen exposure and their ability to release mediators are reduced in treated patients, decreasing the inflammatory response of the tissues [9].

Due to these capacities, AIT reduces the allergic symptoms in a significant fraction of treated individuals and improves their quality of life. However, although AIT reduces the progression of allergy and prevents the development of asthma, it is also associated with frequent local and systemic adverse reactions, which can vary from mild symptoms to severe reactions such as anaphylaxis and, in some cases, life-threatening conditions [13]. AIT also presents other problems such as the variable efficacy of the treatment, difficulty in standardizing vaccines due to the varying potency of the different allergenic composition of the vaccines currently available, the presence of other allergenic proteins present in natural allergen extracts, the regimen of administration, or the lengthy duration of the treatment [13, 14].

2. GUIDELINES AND ROUTES OF ADMINISTRATION: PROS AND CONS

Currently, AIT with whole allergen extracts is administered in clinical practice according to a procedure known as subcutaneous immunotherapy (SCIT) or sublingual drops or tablets (sublingual immunotherapy, or SLIT) [12, 15]. Route selection depends on many factors such as vaccine availability, cost, and patient

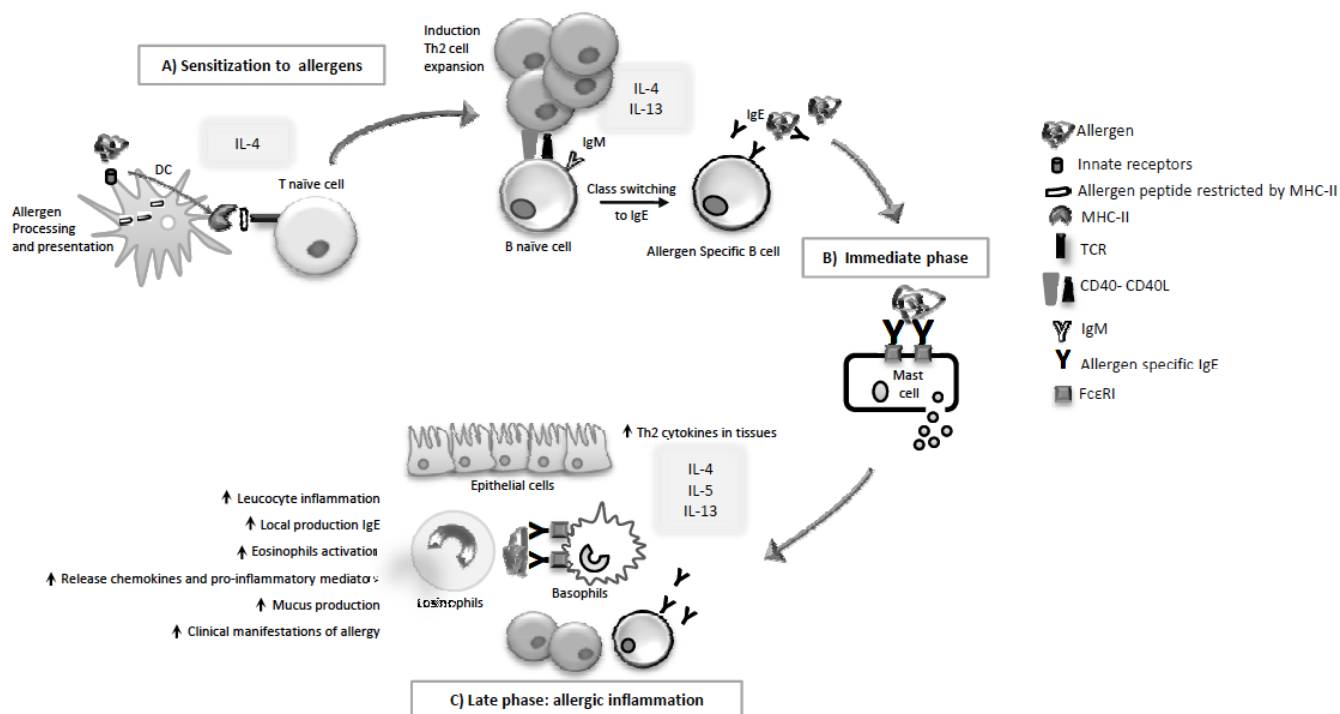


Fig. (1). Allergic immune response. **A) Sensitization phase.** After antigen processing, dendritic cells (DC) present fragments of allergens by MHC-II. T naïve cells recognize them *via* TCR. It induces the differentiation and clonal expansion of allergen-specific T helper 2 with the subsequent production of Th2 cytokines (IL-4 and IL-13). B naïve cells are induced to class switching (IgM to IgE). **B) Allergen specific IgE bound FcεRI** drive to secrete pro-inflammatory cytokines and other chemokines inducing the immediate symptoms of allergic disease. **C) Late phase of the allergic reaction:** after new allergen exposure and under the influence of certain chemokines, allergen-specific cells are reactivated and expanded, effector cells (eosinophils, basophils and mast cells) are also recruited to the tissues and this contributes to the development of clinical symptoms and the persistence of allergic response, which becomes chronic.

characteristics [12]. Both treatments require repeated administrations over a long period of time, which has a negative impact on patient compliance. The optimal duration of treatment is not well defined, though the general recommendation calls for administration over 3-5 years. Overall, SCIT requires an initial build-up phase with increasing doses administered once or twice weekly until the patient reaches a predetermined maintenance dose [16]. SCIT must be administered in a supervised medical facility and be monitored for 30 minutes after the injection, as SCIT may induce a wide range of adverse reactions, local or systemic. The severity of these reactions varies from mild symptoms to life-threatening anaphylaxis and even death [12]. Compared with SCIT, SLIT has a better safety profile. The most common adverse events are local (oropharyngeal pruritus and mucosal swelling), occur in the first few days of therapy, and resolve without medical intervention. Systemic reactions are uncommon [12]. SLIT also allows for home administration but requires daily treatment over several months and an allergen volume

at least 50-100 times greater than SCIT to achieve a similar level of efficacy [17].

Although SCIT and SLIT improve clinical symptoms in many patients, these therapies are not effective against all symptoms and are associated with a high frequency of adverse events related to the allergenicity of the vaccines. Thus, there is a need for safer and more effective vaccines that can reduce the allergenicity of existing AIT while maintaining the capacity to modify the IS response [13].

3. NOVEL AIT APPROACHES

With the purpose of improving the main weaknesses of currently used immunotherapy (to reduce the allergenicity, the side effects, decrease the time of administration, *etc.*) and thanks to the advances in molecular biology and in the knowledge of immune response to allergens, novel approaches are in study. These new approaches include the use of hypoallergens, recombinant proteins, peptides derived from allergens, bacterial DNA motif vaccines, or the modification of the routes

of administration including intranasal, epicutaneous, oral, or intralymphatic [4, 12]. In this review we will focus on describing the main advances in experimental animal models and the clinical trials based on one of the most promising therapy, peptide vaccines.

4. VACCINES BASED ON PEPTIDES

A promising new therapy involves the use of soluble allergen fragments of variable lengths, which could solve of the main problems facing conventional AIT. This approach also offers additional advantages, such as high stability, ease of purification, standardization, and low production cost [18].

Peptide vaccines are designed using a strategy based on the primary structure of the allergen. Thus, there are 2 types of peptides depending on the length of the fragments and their capacity to produce tolerance: IgE-mediated peptides and T-cell peptide–SPIRE (Fig. 2).

4.1. IgE-mediated Peptides

IgE binding to allergens is dependent on the folded tertiary structure of the allergen. Vaccines designed using these peptides are the result of the synthesis of long peptides (20–40 amino acids) that correspond to the B-cell epitopes of the allergens, though they are usually synthesized with minor modifications or linked to a protein carrier to modify the humoral response. Use of these B-cell epitopes aims to induce a non-inflammatory allergen-specific IgG4 (Fig. 2A) or IgA response [4, 14].

4.2. T-cell Peptide Vaccines–SPIRE

The use of short synthetic peptides (derived from T-cell epitopes), with a length of 10–17 amino acids, is based on the lack of conformational B-cell epitopes which could indirectly promote production of non-inflammatory antibodies such as IgG4 or IgA [13]. These short synthetic peptides are designed to be recognized by MHC class-II molecules on APC. These peptides induce Treg cells, possibly triggering the production of IL-10, TGF- β , an induction of Foxp3 expression, and the increase of Th1 response (Fig. 2B). Also, their small size does not permit binding to IgE-Fc ϵ RI on effector cells, so they are not able to cross-link IgE. This provides an advantage over the whole extracts used in clinical practice by potentially reducing the risk of IgE-mediated allergic reactions such rhinitis, asthma, and pruritus [19].

Identifying CD4⁺ immunodominant T-cell epitopes (regions of antigens that are most extensively recognized by antigen-specific T cells) is essential to the de-

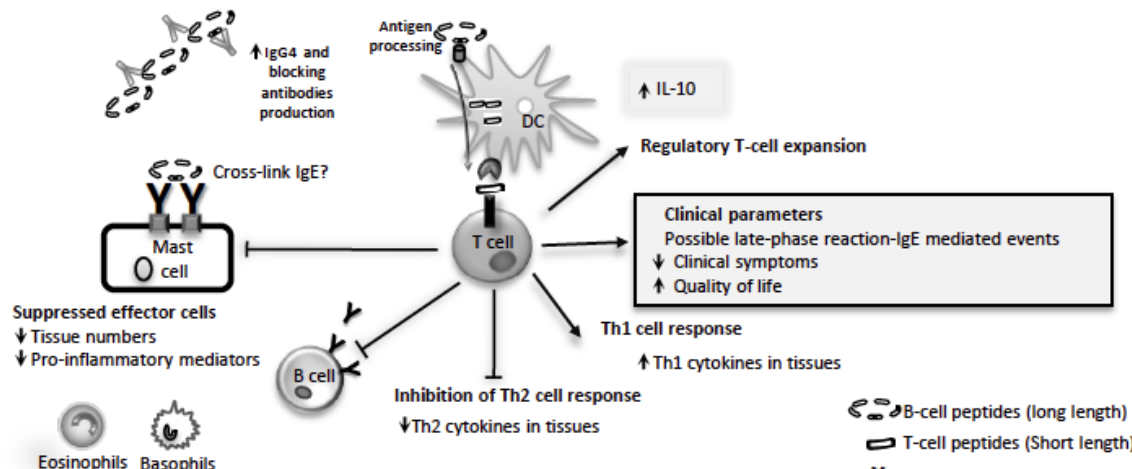
sign of peptide vaccines. Identification can be performed by experimental or predictive strategies. The former uses peripheral blood mononuclear cells (PBMCs) proliferation assay, which consists of locating the main T-cell epitopes by assays of T-cell proliferative response of PBMCs from patients and healthy controls after stimulation with a battery of synthetic peptides (usually overlapping peptides) covering the total length of the antigen molecule. T-cell epitope mapping obviously requires knowledge of the allergen sequence, as it is needed for peptide synthesis. These peptides, in addition, tend to range from 10 to 17 amino acids, because this is the usual length of human leukocyte antigen (HLA) class-II-processed molecules [4, 20]. The other approach, predictive strategy, is based on the use of specific software that predicts the T-cell epitopes according to the motifs recognized by HLA class-II receptors. As has been reviewed by Nielsen *et al.* [21], the predictive performance for MHC-II remains significantly lower than for MHC-I. However, recent progress has been made, and many databases have been assembled to describe the binding specificities for MHC molecules. The 2 main sources of data are the SYFPEITHI database of MHC ligands and T-cell epitopes [22], and the immune epitope database (IEDB) [23]. A limited number of methods are publicly available for quantitative MHC class-II prediction, namely the ARB [24], SVRMHC [25], MHC pred [26], and Net MHCII [21].

Importantly for therapy safety, all candidate T-cell epitope peptides must be tested both alone and in combination to ensure their inability to bind and cross-link inflammatory cell-bound IgE. A convenient and reliable assay to assess clinical and functional relevance of IgE reactivity is the basophil activation test [27]. The use of these non-cross-linking IgE peptides reduces the potential to cause either local injection reactions or systemic reactions, which is one of its main differences with respect to conventional AIT [16]. Recently, this kind of peptide has been called SPIRE (synthetic peptide immuno-regulatory epitopes) because of its capacity to induce immunotolerance [16, 19].

4.3. Tregpitopes

In 2008, regulatory T-cell epitopes (Tregitopes) were defined as peptides with special characteristics. This definition emphasized such traits as a pattern of “promiscuous binding to major histocompatibility complex”, among others. This ability leads Tregitopes to suppress effector T-cell response, to induce a regulatory T-cell profile with IL-10 secretion, and to up-regulate other Treg-associated cytokines and chemokines. Tregpitopes are conserved

A) B-cell peptides



B) T-cell peptides

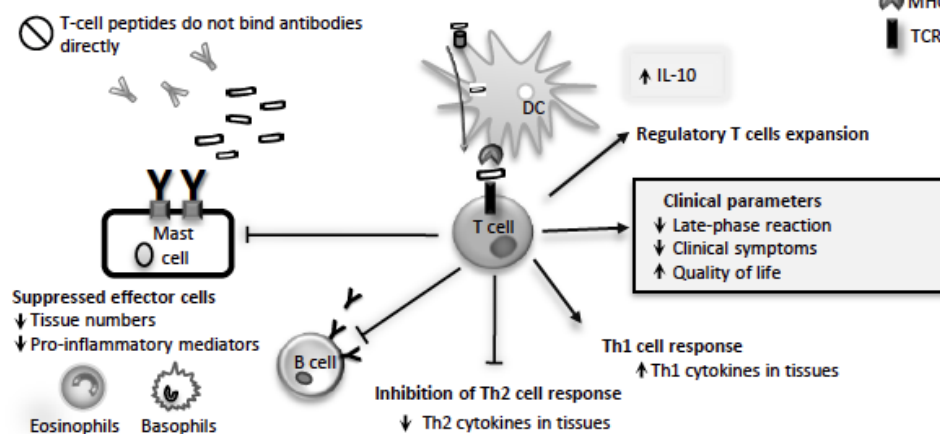


Fig. (2). Effects of peptide immunotherapy on clinical and immunology parameters. **A)** B-cell peptide leads to direct generation of IgG4 antibodies. These peptides maintain the conformational structure because of the long length (20-40aa), blocking the bind to specific IgE. **B)** T cell peptides due to their short size (10-17 aa) are not able to bind antibodies directly, so effector cells are not activated. In both cases, **A)** and **B)** peptides are presented by APC or DC to T-cells with costimulatory signals, leading to the induction of mixed Th1-Treg response increasing the ratio of Th1 to Th2 cytokines. The high secretion of IL-10 decreases eosinophil, basophil, and mast-cell recruitment to the tissues and the ability to release mediators. These facts reduce clinical symptoms and improve the quality of life of patients.

across IgG isotypes and mammalian species, and their potential for use in therapies for autoimmune diseases is now being explored [28, 29]. Most studies suggest that Tregpitopes may be used to treat many autoimmune diseases [30], thus making this type of peptides relevant to cure allergies, as has been pointed by Mazer B *et al.* [31].

Below we summarize the main advances in peptide immunotherapy, from animal models to clinical trials. This technology has been translated to clinical practice in recent years, achieving significant efficacy in a limited number of allergies. Here, we highlight the study

of house dust mite (HDM) allergy, cat allergy, bee-venom allergy, and *Oleaceae* pollinosis.

5. ANIMAL EXPERIMENTAL MODELS

Prior to clinical trials of therapeutic peptides, pre-clinical experimental models of allergies have been developed to validate the strategy of peptide immunotherapy, measure its efficacy, and explore the mechanisms involved in tolerogenic response. Table 1 contains a summary of the main results in peptide therapy using animal models.

Table 1. Summary of the most relevant peptide immunotherapy in allergic animal models.

Allergen	Treatment Based on Peptides			Animal Model	Main Results	Reference
	Peptide Derived from	Size	Administration Route			
Der p 1	Major T cell epitope Der p 1 (111-139)	29	IN	HDM Mice allergic model	Prevent the immunization (seven days after) with the whole Der p 1 by subcutaneous injection Peptide induced T cell tolerance, T cell anergy and changes in antibody response to Der p 1.	[34]
					Peptide induced a rapid but transient activation of MHC class II restricted CD4+ Peptide induced a strong transient CD4+ T cell response	[35]
	A mixture of 2 peptides		IN	Mice co-sensitized to HDM and OVA	Peptide immunotherapy reduced AHR, Ovalbumin-specific T-cell proliferation, Th2-cell numbers and pro-inflammatory cytokines. Modulation of the response to OVA was associated with induction of IL-10 production and Foxp3 expression, but not with an increase of IL-35 production	[36]
	Der p 1 (55-69)	15				
	Der p 1 (149-167)	19				
Fel d 1	A mixture of 2 long peptides		SC	Cat-mice allergic model	Peptides inhibited Th2 clonal expansion, as well as decreased the production of IL-2 and allergen specific IgG	[38]
	Fel d 1 chain I (7-33)	27				
	Fel d 1 chain I (29-55)	27				
	Fel d 1 chain I (29-45)	16	ID	HLA-DR1 transgenic cat-mice allergic model.	Improvement in AHR was associated with reduced recruitment, proliferation, and effector function of allergen-specific Th2 cells in lung tissue	[39]
Api m 1	3 long overlapping peptides		IP IN	Murine model of hypersensitivity to PLA2	This therapeutic approach increased specific IgG2 and decreased allergen-specific IgE levels T cell cytokines secretion was characterized by a shift from a Th2 to a Th1 profile. Peptides were able to provide an efficient protection in sensitized mice challenged with the native allergen	[41, 42]
	Api m 1 (1-60)	61				
	Api m 1 (47-99)	52				
	Api m 1 (90-134)	44				
Ole e 1	Ole e 1 (109-130)	22	IN	Mice were sensitized to Ole e 1	Pretreatment of mice with the peptide led to suppress serum specific IgE, IgG1 and reduced proliferative T-cell response and Th2-cytokine production. Pathologic alterations of the lung associated with AHR were also reduced	[47]
	Ole e 1 (109-130) binding PGLA microparticles				Peptide T-PGLA MP prevented systemic allergic immune response <i>via</i> Th1 activation	[48]

IN: intranasal; SC: subcutaneous; ID: intradermal; IN: intraperitoneal, PGLA: Polylactide-co-glycolide.

5.1. Der p 1 Peptides

HDMs and their waste products are one of the predominant sources of indoor allergens. HDM allergens are found in the indoor environment throughout the

year under certain conditions of temperature and relative humidity [32]. HDM sensitization is very common and is associated with perennial rhinoconjunctivitis and asthma, and also contributes to atopic eczema and other

allergic skin diseases. It has been described that up to 50% of individuals with asthma are sensitized to mites, being the most frequently responsible species *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* [32, 33].

One of the first peptide treatment studies was carried out in an HDM sensitization model. Naïve mice were treated intranasally with peptides corresponding to the immunodominant epitopes (111-139 aminoacids) of one of the major allergens (Der p 1), a cysteine protease present in the intestinal tracts of *Dermatophagoides pteronyssinus*. Following treatment (7 days after), the mice were not sensitized against whole Der p 1 administered by subcutaneous injection. Peptide treatment induced T-cell tolerance, T-cell anergy, and changes in the antibody response to Der p 1 [34, 35].

Another interesting study involved treatment with the 2 peptides Der p 1⁽⁵⁵⁻⁶⁹⁾ and Der p 1⁽¹⁴⁹⁻¹⁶⁷⁾ in a mouse model co-sensitized to HDM and ovalbumin. This mixture of peptides was able to decrease airway hyperresponsiveness, T-cell proliferation, pro-inflammatory cytokines, and tissue eosinophilia. This increase of tolerance was associated with the induction of IL-10 production and Foxp3 expression [36].

5.2. Fel d 1 Peptides

Sensitization to cat is a common cause of allergic disease all over the world, with a prevalence of 10–15% in the adult population. The most important and potent cat allergen in humans is the secretoglobulin Fel d 1, a protein with unknown function produced by the skin and the salivary and lacrimal glands of the cat. Eighty to 95 percent of cat-allergic patients have specific IgE antibodies against this protein. Fel d 1 has been extensively studied through biochemical and immunological characterization [37].

Over the years, T- and B-cell epitopes have been analyzed, providing evidence of the utility of peptides derived from Fel d 1 in mouse models as a therapeutic tool [37]. In this research, the treatment of mice sensitized to Fel d 1 with 2 long peptides (containing some, but not all, of the T-cell epitopes from Fel d 1 chain 1) inhibited Th2 activation and decreased the production of IL-2 and allergen-specific IgG1 and IgG2a. The ability to induce tolerant T-cell responses with subcutaneous injections suggested a practical approach to the treatment of human diseases with peptides containing these T-cell epitopes [38]. More recently, Campell *et al.* [39] developed a novel HLA-DR1 transgenic mouse model of asthma. Mice were sensitized with recombinant Fel d 1, inducing allergic lung inflammation. A

low dose (1 µg) of peptide Fel d 1⁽²⁹⁻⁴⁵⁾, known to bind the transgenic HLA, was able to ameliorate allergic inflammation, reduce eosinophilia, improve lung function, and reduce the number of Th2 cells infiltrating the lung tissue. These effects were observed to be IL-10-dependent, providing evidence of the ability of peptides of to re-establish immune tolerance.

5.3. Api m 1 Peptides

Bee sting causes intense local allergic reactions (erythema, urticaria, or angioedema) and may induce systemic reactions with generalized erythema or more severe respiratory and vascular compromise, with the inherent risk of anaphylaxis in predisposed individuals. The main allergen is phospholipase A2 (PLA2), also called Api m 1, an enzyme that hydrolyzes membrane phospholipids to produce lysophospholipids and arachidonic acid [40]. Studies based on peptide therapy demonstrated the usefulness of this approach to induce tolerance against the allergen. In a murine model of hypersensitivity to Api m 1, it was demonstrated that intraperitoneal administration of 3 allergen-derived long overlapping peptides (Api m 1⁽¹⁻⁶⁰⁾, Api m 1⁽⁴⁷⁻⁹⁹⁾, Api m 1⁽⁹⁰⁻¹³⁴⁾) modulated an established IgE response. This peptide treatment modified cytokines secretion and humoral response. Also, in the same study it was demonstrated that these peptides could be used as a prophylactic treatment, which explains their capacity to modulate B- and T-cell response, preventing the development of an Api m 1-induced allergic response [41]. Similar results were observed with intranasal administrations of these long peptides, showing that the nasal route could be an alternative to conventional immunotherapy without the inherent risk of systemic anaphylactic reaction involved with other administration routes [42].

5.4. Ole e 1 Peptides

Olive-pollen allergy is one of the most important respiratory allergies in the Mediterranean countries and some areas of North America [43]. Olive pollen induces mainly nasal and conjunctival symptoms, although it may also induce exacerbation of asthma in areas with high antigenic load [44]. Their major allergen, Ole e 1, is a glycoprotein that shows a high degree of sequence homology and IgE crossreactivity to the main allergens in other *Oleaceae*-family pollens (lilac, ash, and privet) [45]. The use of peptides derived from Ole e 1 has been studied in a mouse model, using intranasal pretreatment with a peptide Ole e 1⁽¹⁰⁹⁻¹³⁰⁾ containing a dominant T-cell epitope of Ole e 1 [46] one week prior to sensitization with the whole allergen.

This treatment demonstrated the potential to protect mice against olive-pollen sensitization associated with an increase of INF- γ and IL-10 [47]. These results suggested that this peptide alone could be used as prophylactic treatment. Additionally, the same group demonstrated that the intranasal immunization with peptide-PGLA (polylactide-co-glycolide) microparticles was effective in the prevention of subsequent allergic sensitization to Ole e 1 [48].

The main conclusions of these peptide therapy studies in animal models show how some regions of the major allergens in HDM, cat, bee venom and *Oleaceae* pollen are able to change the immunological and clinical responses using different routes of administration, laying the bases for their prophylactic and therapeutic use in order to treat these sensitizations in more specific fashion.

6. TRANSLATIONAL AND CLINICAL STUDIES OF PEPTIDE-BASED ALLERGY THERAPIES

Advances in experimental animal models make it possible to transfer peptide immunotherapy into the human studies (summarized in Table 2). Translational studies and clinical trials have been performed to evaluate the safety, efficacy, and clinical advantages of this approach in the allergies described above.

6.1. Derp 1 Peptides

A phase I clinical trial demonstrated the safety and effectiveness of a synthetic Der p 1 peptide (16 amino acids) coupled with a virus-like particle derived from bacteriophage QB (QB- Der p 1). Twenty-four healthy volunteers were randomized to 4 treatment groups depending on dose (10 or 50 mg of QB- Der p 1) and the route of application (intramuscular or subcutaneous). After boosting immunizations one and 3 months later, antibody titers were measured several times (1, 3, 4, 6, 12, and 18 months). The vaccine was well-tolerated; the 50-mg group also increased the specific IgG titers independently of the route of administration [49]. In another clinical trial, 40 HDM-allergic subjects received HDM-peptide antigen desensitization (HDM-PAD) intradermally, using a mixture of T-cell epitopes also known as Toleromune® HDM. Subjects were randomized to 5 groups (0.03, 0.3, 1.0, 3.0, and 12 nmol of peptides), with 4 administrations weekly; 2 subjects received placebo. Eighteen to 22 weeks after the treatment, conjunctival provocation test (CPT) and early- and late-phase skin response (EPSR and LPSR) were remeasured. Patients had no serious adverse events. CPT scores were reduced by between 16.7% and 41.4%, without changes in the placebo group. The de-

crease in EPSR and LPSR score (median % change of -39.19% and -51.19%, respectively) in the 4×3 nmol administration group after the treatment showed that these peptides have biological activity, and the treatment is safe and well-tolerated by the patients [50]. This group also evaluated the potential of peptides with regulatory capacities called HDM-SPIRE using an environmental exposure chamber (EEC). Eighty HDM-allergic subjects were divided into 4 groups depending on dose (placebo, 3, 6, 12 nmol of HDM-SPIRE; 4 injections in 4 weeks). EEC challenge consisted of 3 consecutive days of 4-hour allergen exposures (Der p 20-120 ng/m³) at baseline and after 19, 36, and 50 weeks. Total rhinoconjunctivitis symptom score (TRSS) was recorded every 30 minutes on a scale of 0–24. The 12-nmol group had the best results as measured by major decreases of TRSS. The reduction of symptoms persisted 1 year after the treatment [51]. Following this study, the effect of the treatment was evaluated with the administration of 4 injections $\times$ 12 nmol of HDM-SPIRE peptides over 12 weeks, 2 years after the end of the treatment. A total of 72 patients who had previously participated in a one-year randomized study were recruited a year later. Treated patients showed a 22.7% or greater improvement in TRSS relative to the placebo group, in both the EEC and in the field [52].

To date, peptide immunotherapy for the treatment of HDM has been tested in phase 2 clinical trials with promising results. As we have summarized, safety and tolerance were good, and clinical symptoms were reduced significantly. Also, short regimen of treatment was able to induce long-term tolerance.

6.2. Fel d 1 Peptides

Since the 1990s, several studies have evaluated the safety and efficacy of different peptides from Fel d 1 and the capacity of these peptides to treat the disease.

One of the first clinical trials was conducted by Norman P *et al.* [53]. The study analyzed the therapeutic potential of 2 long peptides derived from the 2 chains of Fel d 1 protein, which contain the dominant epitopes IPC1/IPC2 (ALLERVAX CAT). The study population included 95 cat-allergic patients randomized into 4 groups: placebo, and 7.5, 75, and 750 μ g of peptide mixture per injection. Treatment administration involved 4 subcutaneous injections for 4 weeks. The high-dose group showed an improvement in nasal and lung symptoms after 6 weeks of treatment, although early adverse events occurred frequently. The immediate reactions appeared to be IgE-mediated, and it was suggested that the large peptides employed retained the

Table 2. Summary of human studies of peptide immunotherapy.

Allergen	Treatment Based on Peptides			Study Design Number of Subjects	Main Findings	Adverse Events	Reference
	Peptides	Regimen of Administration	Route				
Der p 1	Peptide from Der p 1 coupled to a VLP derived from the bacteriophage Qb (Qb-Der p 1)	4 treatment groups. Depending on the doses and the <i>via</i> of administration 10 or 50 mg of Qb-Der p 1 intramuscularly 10 or 50 mg of Qb-Der p 1 subcutaneously	IM and SC	Phase 1 24 HDM allergic patients	The 50mg group increased the specific-IgG titers independently of the route of administration	Qb-Der p 1 was well tolerated	[49]
	HDM-PAD (peptide antigen desensitization)	5 treatment groups (0.03, 0.3, 1.0, 3.0 and 12 nmol of peptide) and placebo; 4 administrations 4-weeks apart	ID	Phase 2a 42 HDM allergic patients	Treatment reduces the EPSR, LPSR score in the 4x3 nmol group CPT score was reduced in all treatment regimen	HDM-PAD was safe and well tolerated. There were no serious adverse events	[50]
	HDM SPIRE	4 treatment groups, placebo, 3, 6 and 12 nmol of HDM SPIRE; 4 injections in 4 weeks	–	Phase II b 80 HDM allergic patients	The 12 nmol group was the group with the most ameliorates regarding to the major decrease of TRSS. The reduction of symptoms persisted 1 year after the treatment.	No adverse effects were occurred	[51]
	HDM SPIRE	2 treatment groups. 12 nmol of HDM SPIRE and placebo; 4 injections in 4 weeks	–	Phase II b 72 HDM allergic patients	Two years after the start of treatment patients improved their TRSS	No adverse events were occurred	[52]
Fel d 1	ALLERVAX CAT treatment: two long peptides derived from the two chains of Fel d 1 protein	4 treatment groups. Placebo, 7.5 µg, 75 µg, and 750 µg of peptide per injection Patients received an weekly injection for 4 week	SC	Double-blind placebo controlled trial 95 cat allergic patients	750 µg group reduces the nasal and lung symptoms scores compared with baseline	Frequent early adverse events were occurred	[53]
		2 groups of treatment, 250 ug or placebo, weekly injected for four consecutive weeks	SC	Double-blind, parallel-group study 42 cat allergic patients	This study could not demonstrate a positive clinical response with these peptides	Treated patients had some adverse events	[56]
		3 groups of treatment, placebo, 75 µg/dose and 750 µg/dose. Injections were administered twice weekly for 2 week and, a dose was re-administrated 4 months later	SC	133 cat allergic patients	Treatment with high doses of peptides improved pulmonary functions in patients with reduced baseline forced expiratory volume in 1 second (FEV ₁) compared with placebo group	Frequent early adverse events were occurred, both in placebo and treatment group.	[47]
	12 short peptides derived from Fel d 1	2 injections of peptides (1, 2.5 and 5 µg) and the whole allergen were studied	ID	24 cat allergic patients	Patients who received 5 µg of each peptide reduced the early and the late-phase (6-h) skin reactions induced by the whole allergen. The treatment had the ability to alter the T cell in vitro response	No adverse events were occurred	[59]
		2 groups: placebo and 12 Fel d 1 peptides (total dose of 90 µg of each peptide in increasing divided doses)	ID	placebo-controlled, double-blind clinical trial 24 cat allergic patients	Peptide treatment reduced late-phase skin reaction	No adverse events were occurred	[60]

(Table 2) contd....

Allergen	Treatment Based on Peptides			Study Design Number of Subjects	Main Findings	Adverse Events	Reference
	Peptides	Regimen of Administration	Route				
	11 short peptides derived from Fel d 1	Incremental doses of Fel d 1 peptides (0.1, 1, 5, 10, 25 µg; amount of each peptide in an 11-peptide mixture) were given at two-weekly intervals	ID	Double-blind placebo-controlled Eight allergic patients	Fel d 1 peptides decreased airway hyper-responsiveness and inhibited the late phase cutaneous reaction to whole cat allergen	The treatment was in general well tolerated	[61]
	12 short peptides derived from Fel d 1	2 groups of treatment: Group 1 who received incremental doses of Fel d 1 peptides (0.1, 1, 1, 5, 10 and 25 µg) totalling 42.1 µg of each peptide. Group 2 who received a total of 291 µg (1, 5, 10, 25, 50, 100 and 100 µg) of each peptide.	—	A total of 20 cat allergic subjects	Peptide therapy modulates the proliferative response of PBMC from allergic patients against the specific allergen and changes the profile of cytokine responses, characterized by IL-10 induction and IL-5 suppression	—	[62]
	12 short peptides derived from Fel d 1	2 groups: placebo and 12 Fel d 1 peptides (total dose of 300 µg of each peptide in increasing divided doses)	ID	Sixteen cat-allergic asthmatic subjects	In peptide treatment group there was significant improvements in asthma quality of life	None of the subjects developed important adverse events	[63]
	Cat-PAD A mixture of seven peptides	Placebo and subcutaneous or intradermal injections of 0.03, 0.3, 3, 12 or 20 nmol Cat-PAD	ID	Phase IIa clinical trial 88 cat allergic patients	Cat-PAD was safe and well tolerated when administered by means of intradermal injection at doses of up to 12 nmol and when administered by means of subcutaneous injection at doses of up to 20 nmol.	No severe adverse events were occurred	[64]
	Cat-PAD	Placebo or 4 different regimens with Cat-Pad 4 injections x 3nmol, 2 weeks apart 4 injections x 6nmol, 2 weeks apart 4 injections x 3nmol, 4 weeks apart 8 injections x 3nmol, 2 weeks apart	ID	121 cat allergic subjects	Significantly greater ameliorations in rhinoconjunctivitis symptoms, evaluated in a EEC, in the group receiving 8 administrations of 3 nmol 2 weeks	No serious adverse events were reported	[58,65,66]
	Cat-PAD	Placebo or 8 injections of Cat-PAD: 2 regimens of administration 8 injections x 3 nmol- 2 weeks apart 4 injections x 6 nmol- 4 weeks apart	ID	Randomized, placebo-controlled study using EEC 202 cat allergic subjects	Treatment with the 4x6 nmol regimen showed the clinical improvements. The efficacy persisted after a year of treatment	No severe adverse events were occurred	[67]
Api m 1	3 T cell-immunodominant peptides derived from Api m 1. PLA (45-62), PLA (82-92), PLA (113-124)	2 groups of treatment: 10 bee venom allergic patients received conventional immunotherapy 5 allergic patients received incremental doses of an equimolar mixture of peptides.	SC	Exploratory study/ 15 bee venom allergic patients	The ratio of PLA-specific IgG4/IgE was increased. Peptides inhibited the proliferative response and the secretion of Th2 cytokine (IL-4, IL-5) and Th1 profile (IL-2 and INF-γ)	No relevant adverse events were noticed during the peptides treatment or after PLA skin test	[71]
	Peptides derived from Api m 1 (44-60 aa). PLA2 (1-60), PLA2 (47-99), PLA2 (90-134)	2 groups: Placebo and Peptide mixture that was administrated to 9 bee venom allergic patients in acumulative doses of 753.1 µg, within 3 hours. 7 patients were re-injected at days 4, 7, 14, 42 and 70 with a maintenance dose of 300 µg.	SC	16 bee venom allergic patients	T cell proliferation increased in peptides treated group until day 14 to progressively decrease. IL-10 and IFN-γ cytokines increased their levels reaching the maximal peak at day 42. Serum anti-PLA2 IgG4 response was enhanced.	Peptides were well tolerated. Local mild symptoms were observed in two patients	[72]

(Table 2) contd....

Allergen	Treatment Based on Peptides			Study Design Number of Subjects	Main Findings	Adverse Events	Reference
	Peptides	Regimen of Administration	Route				
	Peptides derived from Api m 1	A mixture of peptides was injected with nine intradermal doses at weekly intervals. The accumulative dose was 431.1 µg.	ID	12 bee venom allergic patients	This treatment reduced the proliferation index of antigen specific stimulated PMBCs, associated with a decrease in IL-13 and INF-γ production. Regulatory parameters were increased: IL-10 and a modest up-regulation of SOCS3 gene	Peptides were well tolerated and no treatment-emergent adverse events were occurred.	[73]
Ole e 1	15 overlapping peptides derived from Ole e 1	PBMC from healthy controls and olive-pollen allergy were stimulated with each peptide	—	Exploratory study T-cell epitope mapping	This study identified five peptides with different response pattern. Peptides 2 and 3 with possible immune-modulating properties (high secretion of IL-10), and peptides 10, 12 and 13 as immunodominant epitopes	—	[46, 75]
	5 peptides derived from Ole e 1 peptides 2 and 3 peptides 10, 12 and 13	PBMC from healthy controls and olive-pollen allergy were stimulated with peptides or the whole olive-pollen extract	—	Translational study 15 olive-pollen allergic patients and 14 healthy controls	Changes in gene expression profile, cytokine secretion and PBMC proliferation were observed with peptides 2 and 3 improving the immunoregulatory response	—	[76]
	5 long peptides (32-36 aa) bound to the carrier molecule keyhole limpet hemocyanin	The IgE and T-cell reactivity of the peptides was compared with that of Ole e 1 by means of dot blot experiments, as well as ELISA.	—	Translational study 63 sera and 6 PBMCs of oleaceae-sensitized patients	Two peptides from N-terminus of protein induced much lower lymphoproliferative response.	—	[77]

Adapted from [79]. CPT: conjunctival provocation test, EPSR: early phase skin response, HDM: house dust mite, ID: intradermal, IM: intramuscular, LPSR: late phase skin response, PLA: phospholipase, SC: subcutaneous, TRSS: total rhinoconjunctivitis symptom score, VLP: virus-like particle.

conformational epitopes. Mechanistic studies by *ex vivo* T-cell response assays indicated that this peptide therapy induced a dose-dependent decrease of IL-4 production, suggesting a shift in T-cell phenotype [54, 55]. Another study tested these peptides in a cohort of 42 cat-allergic patients receiving subcutaneous injections of peptides (250 µg/dose) or placebo weekly for 4 weeks. However, this study could not demonstrate a positive clinical response with these peptides: this peptide immunotherapy did not reduce immediate- or late-phase skin reactivity to cat extract containing Fel d 1, and no changes were observed in the PBMCs cytokine secretion between the 2 groups (peptide treatment *vs* placebo) [56]. A larger clinical trial of ALLERVAX CAT treatment was performed in a multicentre, double-blind, placebo-controlled study of 133 cat-allergic patients chronically exposed to cats. Patients were randomized into 3 groups (placebo, 75 µg/dose, and 750 µg/dose). Subcutaneous injections were administered twice weekly for 2 weeks, and a dose was re-administrated 4 months later. Three weeks after therapy, patients were clinically examined. Although frequent adverse events were recorded both in the treat-

ment and placebo group, these were mild and easily controlled. Treatment with high doses of peptides improved pulmonary function in patients with reduced baseline forced expiratory volume in 1 second (FEV₁) compared to the placebo group [57].

Years later, the strategy was changed and the studies were focused on the use of small-size peptides (13-17 amino acids) from Fel d 1 derived from T-cell epitopes, and presumably without the ability to cross-link allergen-specific IgE. The route of administration was also modified, this second generation of epitope-based allergen peptides was administrated intradermally instead of subcutaneously to aid access to APC [27, 58]: one of the first studies of this second generation of cat peptides was developed by Oldfield *et al.* in 2001 [59]. In this analysis, the use of 2 intradermal injections of 12 overlapping peptides and the whole allergen was studied in a cohort of 24 patients. Patients who received 5 µg of each peptide reduced the early- and the late-phase (6 h) skin reactions induced by the whole allergen. Furthermore, the treatment had the ability to alter the T-cell *in vitro* response. They observed a significant decrease in peptides and whole allergen-

induced proliferation of PBMCs associated with a decrease of IL-4, IL-13, as well as an unexpected reduction in IFN- γ levels [59]. Similar results were observed in a placebo-controlled, double-blind clinical trial. Twenty-four cat-allergic patients were randomized into 2 groups, consisting of placebo and 12 Fel d 1 peptides (total dose of 90 μ g of each peptide in increasing divided doses). Outcomes were measured at baseline, 4–8 weeks, and 3–9 months. The active treatment group reduced their late-phase skin reactions between baseline and the rest of measures. However, these differences were not statistically significant. Proliferative PBMC assays were reduced in the group treated with peptides, and the production of IL-10 was enhanced. These data suggested the potential role of regulatory T-cells in tolerance induced by peptides [60]. Higher doses of synthetic peptides demonstrated that this treatment enhances the Th1 response (increase in the number of CD4+, INF- γ +, and CD25+ cells) in the cutaneous late-phase reaction sites, suggesting a modification in the Th2:Th1 balance [61]. Additionally, peptide therapy provides evidence of an induction of regulatory and suppressive CD4+T-cell responses. Peptide therapy modulates the proliferative response of PBMCs from allergic patients against the specific allergen and changes the profile of cytokine responses, characterized by IL-10 induction and IL-5 suppression [62]. These findings were associated with the ameliorations of clinical symptoms, including asthma and rhinitis [63].

More recently, the main objective of studies has been to generate a prototype formulation of a peptide vaccine defined by the major T-cell epitopes. Seven peptides that formed this vaccine (formerly called Cat-PAD, peptide antigen desensitization) were described based on their binding to MHC class-II, assays of proliferation, and analyses of profile of induced cytokines. In a phase IIa clinical trial, this intradermal vaccine was identified as safe and well-tolerated by patients, as it was shown that they did not induce histamine release from basophils, with no adverse events recorded [64].

In subsequent trials, the clinical efficacy of the Cat-PAD vaccine was demonstrated in an EEC challenge delivering controlled levels of the allergen. Patients were exposed to the allergen for 3 hours on 4 consecutive days in the EEC. The response was analyzed in 121 subjects with 4 different regimens of treatment. The main differences were found between dose levels and dose regimen (4 or 8 administrations, 3 or 6 nmol per injection and 2 or 4 weeks apart). Subjects returned 18–22 weeks after the beginning of the treatment, and clinical symptoms were evaluated in the EEC chal-

lenge. No significant treatment-emergent events were evidenced. The researchers observed significantly greater ameliorations in rhinoconjunctivitis symptoms in the group receiving 8 administrations of 3 nmol 2 weeks apart when compared to placebo [58, 65, 66]. These findings make it possible to evaluate the effect of treatment over time. With this purpose, the clinical symptoms were evaluated in an EEC, comparing the longer administration, with the minor dose (8 administrations, 3 nmol dose) vs the short administration with the high dose (4 administrations, 6 nmol dose), 4 weeks apart. The results showed persistent treatment effects with significant improvements in rhinoconjunctivitis symptoms one year and 2 years after the start of the treatment [58, 67–69].

Cat-PAD is the most highly developed vaccine using this strategy. A Phase III clinical trial is now under way [58]. Real advances in peptide immunotherapy show promise for the treatment of cat allergy in the future. Here we have summarized how clinical data from a series of studies show that Cat-PAD is able to significantly reduce allergic rhinoconjunctivitis symptoms after a short course of treatment with long-lasting post-treatment efficacy. Furthermore, this therapy could solve the main difficulties of conventional cat AIT, that is, the suppression of treatment due to the high rate of adverse events. However, the data obtained need to be confirmed in large-scale, controlled clinical studies in a real-life setting [58].

6.3. Api m 1 Peptides

The first study of peptide immunotherapy in bee-venom allergy compared treatment with 3 T-cell-immunodominant peptides derived from Api m 1 (PLA^(45–62), PLA^(82–92), and PLA^(113–124)) and conventional bee-venom immunotherapy [70]. A group of 10 bee-venom-allergic patients received conventional bee-venom immunotherapy, and another group composed of 5 allergic patients received incremental doses of an equimolar mixture of selected peptides, administered subcutaneously, within 2 months. PLA intracutaneous skin test was performed on the patients and a week later with bee sting. No relevant adverse events were noticed during the peptide treatment or after PLA skin test; only 2 patients had mild systemic allergic reactions after the bee-sting challenge. Humoral immune response (levels of specific IgE and IgG4), PBMC proliferation analysis, and cytokine levels were evaluated after the treatment. IgE and IgG4 levels decreased after the peptide immunotherapy but, after the bee sting challenge (one week later), their levels increased substantially. Interestingly, the ratio of PLA-specific

IgG4/IgE increased. Patients in whom peptide treatment inhibited the suppression of proliferative response also exhibited reduced secretion of the main Th2 cytokine (IL-4, IL-5) and Th1 profile (IL-2 and INF- γ). The study concluded that peptide therapy changed T- and B-cell responses in the same way as conventional immunotherapy [71]. Fellrath *et al.* [72] studied the safety and efficacy of long peptides derived from Api m 1. The peptide lengths varied between 44 and 60 amino acids. The study consisted of a double-blind, placebo-controlled phase I clinical trial. Peptide mixture was administered to 9 bee-venom-allergic patients in a cumulative dose of 753.1 μ g within 3 hours. Seven patients were injected with a maintenance dose of 300 μ g at days 4, 7, 14, 42, and 70. The control group was injected with albumin solution. Overall, the peptides were well-tolerated, although local mild symptoms were observed in 2 individuals. T-cell proliferation increased in the peptide-treated group until day 14 and progressively decreased in the following injections. This pattern of response demonstrated that active tolerance induction occurs after day 42. IL-10 and INF- γ cytokines increased their levels, reaching a peak at day 42. After that, the levels declined until day 80. Serum anti-PLA2 IgG4 response, but not specific IgE levels, was enhanced, likely due to the presence of some intact B-cell epitopes within the long peptides. Three years later, a study showed how a group of peptides derived from PLA2 improved clinical symptoms, and this clinical improvement was associated with a modulation of immunological parameters in a population of 12 allergic volunteers. A mixture of peptides was injected with 9 intradermal doses at weekly intervals. The cumulative dose was 431.1 μ g. Peptides were well-tolerated and no treatment-emergent adverse events were occurred. The late-phase reaction to PLA2 reduced significantly in the peptide-treated group. This treatment also reduced the proliferation index of antigenic specific stimulated PMBC, and the treatment was associated with a decrease in IL-13 and INF- γ production. Additionally, the regulatory parameter IL-10 was increased, and a modest up-regulation of SOCS3 gene was observed [73].

Considering the findings of these studies, the use of peptides derived from Api m 1 could be useful as a novel and safe specific immunotherapy due to their capacity to modulate the immune response in a way that resembled conventional AIT but without introducing new, potentially allergenic compounds.

6.4. Ole e 1 Peptides

Our group is currently attempting to address the possibility of using Ole e 1-derived peptides to treat

olive-pollen allergy using a T-cell epitope strategy. Cárdaa *et al.* carried out a deep search of immunodominant T-cell epitopes on Ole e 1 molecule [46]. Using 15 overlapping dodecapeptides covering the whole Ole e 1 molecule [74], proliferation assays were performed with PBMCs of both control and olive-pollen-allergic subjects. This study identified 5 peptides with different response patterns: peptide 2 (aa11-22) and peptide 3 (aa22-33), mostly recognized by non-allergic subjects; and peptide 10 (aa91-102), peptide 12 (aa109-120), and peptide 13 (aa119-130), with immunodominant properties, which is to say that these were mostly recognized by allergic subjects [46]. Furthermore, the production of the immuno-regulatory cytokine IL-10 was studied in a preliminary work with stimulated PBMCs. Stimulation with peptides 2 and 3 induced higher secretion of IL-10 in PBMCs. In short, since peptides 2 and 3 were more recognized by non-allergic patients, this differential secretion of IL-10 indicates that these immunomodulating peptides may be used as immunotherapy and could be used as SPIRE [75].

Following the same line of research, in 2015 Cardaba's group published an article on a more in-depth study of the effect of this peptide on controls' and allergic-patient PBMCs, inside and outside pollen season. Interestingly, there were differences between both moments, suggesting a different regulatory response to Ole e 1 therapy depending on the allergen environmental load. Thus, in patient PBMCs collected during pollen season, stimulation with peptides 2 and 3 increased IL-10 secretion significantly, compared with the same stimulus outside pollen season. The control group, on the other hand, showed a more similar IL-10 secretion pattern in both moments. These results demonstrated the importance of taking into account the moment of pollen exposure in seasonal allergies when developing an immunotherapy. Th1- and Th2-cytokine secretion was also studied, though they were too low and had no clear differential pattern between peptide treatments in the control and allergic group [76]. Secondly, we studied how Ole e 1-derived peptides affect gene expression in control and allergic PBMCs. While in the control group there was no clear pattern, allergic-subject samples were separated in clusters corresponding to the immuno-regulatory peptides. Focusing on the pathways and biological processes differentially expressed between groups and experimental conditions, PBMCs from olive-pollen-allergic patients stimulated with peptides 2 and 3 showed the lowest differences compared with controls. Furthermore, in a more in-depth functional analysis comparing pathways differentially expressed after peptide 2 and 3 stimulation with

the whole olive-pollen extract and with immunodominant peptides (peptides 10, 12, and 13), 51 regulator genes were identified, and these were highly related to the immune regulatory response to olive pollen in allergic patients, such as DNMT1, LGMN, DAB2, EB13, CD84, FPR3, TREM1, ALOX5, and other cytokines and chemokines. Functional analysis of these genes revealed that clustering was mainly related to the immune response; most of these genes codify proteins implicated in the maintenance of peripheral T-cell-tolerance, suggesting that these genes could be used as immunotherapy targets in the future. What is more, since there is great similarity between olive pollen and other *Oleaceae* family pollens, this new therapeutic tool could be useful in the treatment of many allergies [76].

Finally, the effect of these peptides on patients' PBMCs is still under study in Cardaba's laboratory. It is important to keep in mind that the effect of the peptides depends on the moment of pollen exposure. For this reason, proliferation assays have been performed with both groups of peptides and also with the combination of all of them, before and after stimulation with the whole olive-pollen extract. The aim is to establish whether it would be better to use the combination of peptides during pollen season or before it (results not published).

In 2011, the group led by Valenta studied the IgE-production capacity and the T-cell reactivity of 5 peptides, ranging from 32 to 36 amino acids, compared to the whole sequence of Ole e 1. Rabbits were immunized with non-IgE-reactive, keyhole limpet hemocyanin-coupled peptides of Ole e 1. The results showed that there were 2 peptides from the N-terminus of the protein, which induced much lower lymphoproliferative responses than whole Ole e 1 [77].

Considering the primary advances in the studies of the peptides derived from Ole e 1, both short and long peptides could be useful as immunotherapy, highlighting the importance of the first 22-33 amino acids in the regulation of the IS and their capacity to induce tolerance.

7. FUTURE OF PEPTIDE TREATMENT

Peptide therapy is a new type of treatment for allergic diseases which induces clinical tolerance against allergens. SPIRE for common allergies such as cat, HDM, or bee venom are currently undergoing clinical trials. Combinations of various dosing regimens and routes of administration have been studied, and current results suggest that this therapy is able to maintain

clinical efficacy over the long term with a short treatment course and without relevant adverse events [19, 27]. In fact, SPIRE treatment seems more convenient than current SCIT and SLIT protocols, as it significantly reduces the number and time of administration for achieving similar outcomes [19]. This fact raises optimism that the discipline of allergology is on the brink of a new era in allergen therapeutics [27].

However, this new vaccines approach has several limitations that should be solved in future research. First, peptide selection must be performed while keeping in mind the MHC-binding characteristics of the peptides [78] to ensure that these peptides are able to cover a wide range of population. Second, the low immunogenicity of many short peptides that could require adjuvants or other compounds that may increase the immunological response [79]. Lastly, further studies of peptide combinations are required in order to increase efficacy.

CONCLUSION

Peptide therapy or SPIRE strategy represents one of the most interesting approaches to the most pressing problems of conventional AIT, including the high allergenicity of allergen extracts used in clinical treatments. Clinical trials demonstrated how SPIRE treatment could be useful to cure these diseases and open a new era in allergen therapy. Overall, SPIREs are safe, well-tolerated, and induce long-term clinical tolerance, thus reducing the main symptoms and improving the quality of life of allergic patients. Although the underlying immunological mechanisms are not currently understood, peptide immunotherapy appears to be dependent upon IL-10 and other Treg mediators. However, the modification of the antibody response depends on peptide length, as well as the route of administration and the regimen of administration of the vaccine. It seems that large peptides retain more B-cell epitopes, enabling them to induce specific blocking antibodies directly. More research is needed to better understand the mechanisms of action involved in this kind of therapeutic responses, as well as, following of long-term treatments.

LIST OF ABBREVIATIONS

AIT	= Allergen-specific immunotherapy
APC	= Antigen-presenting cells
CPT	= Conjunctival provocation test
DC	= Dendritic cells
EEC	= Environmental exposure chamber

EPSR	= Early phase skin response
FcεRI	= High-affinity receptor for IgE
HDM	= House dust mite
HLA	= Human leukocyte antigen
ID	= Intradermal
IL	= Interleukin
Ig	= Immunoglobulin
IM	= Intramuscular
IN	= Intranasal
IP	= Intraperitoneal
IS	= Immune system
LPSR	= Late-phase skin response
MHC	= Major histocompatibility complex
PBMCs	= Peripheral blood mononuclear cells
PLA2	= Phospholipase A2
SC	= Subcutaneous
SCIT	= Subcutaneous immunotherapy
SLIT	= Sublingual immunotherapy
SPIRE	= Synthetic peptide immuno-regulatory epitopes
Th	= T-helper
Tregpitopes	= Regulatory T-cell epitopes
Tregs	= Regulatory T cells
TRSS	= Total rhinoconjunctivitis symptom score

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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