

Mechanism of Synthetic Peptide Immuno-Regulatory Epitopes and Their Clinical Efficacy in the Treatment of Allergic Disease

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Abstract

Purpose of review Peptide immunotherapy with synthetic peptide immuno-regulatory epitopes (SPIREs) is a safer alternative to conventional allergen immunotherapy (AIT) owing to the reduced risk of IgE-mediated adverse reactions and shorter treatment schedules. Over the last 5 years, significant advances have been made in evaluating SPIREs for the treatment of allergic rhinitis. This review includes a summary of recent phase II and III clinical trials evaluating the efficacy of SPIREs and a brief discussion on their mechanism of action.

Recent findings To date, SPIREs have been developed for cat, house dust mite (HDM), ragweed, and grass. Phase II clinical trials showed strong reductions in allergic symptoms, with clinical benefit persisting for up to 2 years after treatment initiation. However, subsequent phase III studies of cat and HDM peptides did not meet their primary endpoint due to significant placebo responses. Mechanistic studies reported a shift from effector T helper 2 (Th2) cells to Th1 and regulatory T cells along with reductions in Th2 cytokines and Th2-associated genes in blood immune cells.

Summary Peptide immunotherapy with SPIREs is efficacious in reducing symptoms after only 12–14 weeks of treatment. Symptom relief can last up to 2 years after treatment and requires fewer injections compared to subcutaneous AIT. Revised trial designs should be considered to re-evaluate these potentially useful therapies in phase III evaluations to overcome placebo responses.

Introduction

Allergic diseases including eczema, food allergy, asthma, and allergic rhinitis (AR) are increasing in prevalence and affect approximately 20% of the global population [1]. While symptomatic medications such as antihistamines and corticosteroids are first line and frequently used, allergen immunotherapy (AIT) serves as the only disease-modifying treatment available for allergic disease. The clinical efficacy of AIT was first reported by Noon and colleagues [2]. By administering subcutaneous injections of timothy grass pollen extract, Noon et al. observed an increase in extract strength required to elicit conjunctivital symptoms in grass-allergic patients. Over the last 50 years, many clinical trials have demonstrated the benefit of AIT in reducing allergic symptoms. Two large meta-analyses of studies from nine international biomedical databases reported a reduction in both symptom scores and use of rescue medication following AIT in patients with allergic asthma or AR [3, 4].

Traditional AIT uses natural allergen extracts prepared from raw material such as animal dander and various pollen species [5]. Due to the preservation of native IgE and T cell epitopes, allergen extracts elicit a high frequency of adverse reactions; up to 75% of patients on subcutaneous AIT (SCIT) experience local reactions (e.g., erythema and swelling) with 0.1% of injection visits associated with a systemic reaction (e.g., asthma or rhinitis exacerbation) during treatment [6, 7]. Although extract-based AIT has been proven effective in desensitizing patients in the clinic, the risk of IgE-mediated anaphylaxis is a major concern for both patients and practitioners alike.

To capitalize on the inherent allergenicity of natural allergen extracts, synthetic peptides derived from T cell epitopes of major allergens were developed. Early studies by Norman et al. [8] and Creticos et al. [9] reported a reduction in allergic symptoms following subcutaneous administration of synthetic peptides (21–27 amino acids in length) derived from T cell epitopes of Fel d 1 and Amb a 1, major allergens of cat and ragweed, respectively. However, both products were discontinued due to the high frequency of late-onset reactions such as asthma. The next generation of peptides was named synthetic peptide immuno-regulatory epitopes (SPIREs). SPIREs are shorter than their predecessors (13–17 amino acids in length) and are derived from immunodominant T cell epitopes exhibiting promiscuous binding to major histocompatibility complex (MHC) class II molecules expressed on antigen-presenting cells (APCs). Due to their short length and absence of reactive IgE epitopes, these peptides are unable to cross-link allergen-specific IgE (sIgE), thus strongly reducing the likelihood of IgE-mediated adverse reactions during treatment [10, 11]. Currently, SPIREs have been developed for common environmental allergens including cat, house dust mite (HDM), ragweed, and grass species (Table 1).

The objective of this review is to provide a summary of recent clinical trials evaluating the efficacy of SPIREs as an immunotherapy approach for the treatment of AR. This review will also discuss recent advances in the mechanism of action of SPIREs in the development of sustained allergen tolerance.

Peptide immunotherapy studies with SPIREs

Currently, the best studied SPIRE-based peptide immunotherapy includes a mixture of seven short Fel d 1-derived peptides for the treatment of cat allergy.

Table 1. Summary of clinical trials evaluating the efficacy of SPIREs

Treatment	Study	Dosing schedule and efficacy analysis	Main outcomes
Cat-PAD/SPIRE: mixture of 7 Fel d 1-derived peptides	Phase II with 2-year follow-up [12••, 13••]	12 or 14-week treatment period of 4 intradermal 6 nmol injections every 4 weeks (4 × 6) or 8 intradermal 3 nmol injections every 2 weeks (8 × 3). Efficacy was evaluated over 4 consecutive daily 3-h cat allergen challenges in an EEC	50–54 weeks after first dosing: significant reduction in mean TRSS in 4 × 6 group vs. placebo ($P = .0104$). 4 × 6 regimen was also superior to the 8 × 3 regimen ($P = .0342$) 2 years after first dosing: Trending reduction in mean TRSS in 4 × 6 group vs. placebo ($P = .13$)
	Phase III [14]	4-dose course of 6 nmol (4 × 6) or 2 sequential courses of 6 nmol (8 × 6)	1 year after first dosing: both treatment courses led to comparable improvements in mean combined scores to placebo. 58.5% placebo response was observed
HDM-PAD/SPIRE: mixture of 7 Der p-derived peptides	DBPC-RCT with 2-year follow-up [15•, 16]	12-week treatment period of 4 intradermal 12 nmol injections (4 × 12) or 11 intradermal 12 nmol injections (11 × 12). Efficacy was evaluated over 3 consecutive daily 4-h HDM allergen challenges in an EEC	49–50 weeks after first dosing: significant reduction in mean TRSS only in 4 × 12 group vs. placebo ($P < .05$) 2 years after first dosing: trend towards symptom improvement in 4 × 12 group vs. placebo
	Phase IIb [17]	4 intradermal 12 nmol injections (4 × 12) or 20 nmol injections (4 × 20) every 4 weeks, or 2 sequential courses of 12 nmol (8 × 12)	1 year after first dosing: all 3 treatment courses led to nonsignificant improvements in mean combined scores vs. placebo (all $P \geq .14$). 39.1% placebo response observed
Ragweed-SPIRE: mixture of Amb a 1-derived peptides	PC-RCT [18••]	Dosing schedule not published. Efficacy was evaluated over 4 consecutive, 3-h ragweed allergen challenge in an EEC	18–22 weeks after first dosing: largest reduction in mean TRSS was seen group with highest baseline symptomology vs. placebo ($P < .05$)
	Phase IIb [19]	4 intradermal 6 nmol injections (4 × 6), 8 intradermal 6 nmol injections (8 × 6), or 12 nmol injections (8 × 12). Treatment completed before ragweed pollen season with efficacy evaluated in an EEC	25 weeks after first dosing: after the peak of ragweed pollen season, a nonsignificant reduction in mean TRSS in 8 × 12 group vs. placebo was seen ($P = .149$). Dose-response effect was present across all 3 treatment courses
Grass-SPIRE: mixture of 7 peptides derived from several grass allergen species (Cyn d	Phase II with 1 and 2-year	14-week treatment period of 8 intradermal 6 nmol injections every 2 weeks (8 × 6), 4	25 weeks after first dosing: after the peak of grass pollen season, a significant reduction

Table 1. (Continued)

Treatment	Study	Dosing schedule and efficacy analysis	Main outcomes
1, Lol p 5, Dac g 5, Hol l 5, and Phl p 5)	follow-up [20••, 21•, 22••]	intradermal 12 nmol injections every 4 weeks (4 × 12), or 8 intradermal 12 nmol injections every 2 weeks (8 × 12). Treatment completed before grass pollen season with efficacy evaluated over 4 consecutive daily 3-h grass allergen challenges in an EEU	in mean TRSS was seen only in 8 × 6 group vs. placebo ($P = .0346$) 1 year after first dosing: Trending reduction in mean TRSS only in 8 × 6 group vs. placebo ($P = .0535$) 2 years after first dosing: Trending reduction in mean TRSS only in 8 × 6 group vs. placebo ($P = .113$)

When the 7-peptide vaccine (Cat-PAD, Cat-peptide antigen desensitization) was administered intradermally, cat-allergic participants receiving the 3 nmol dose showed the greatest reduction in late-phase skin responses following an intradermal cat allergen challenge 3 weeks after treatment [23•]. An improvement in overall symptoms was also observed in cat-allergic children after eight intradermal injections of 6 nmol Cat-PAD given 4 weeks apart [24]. Both preliminary studies showed a favorable safety profile for Cat-PAD, only giving rise to localized erythema and mild pain following the injections. In a larger randomized, double-blind, placebo-controlled, phase II study, 202 cat-allergic participants were randomized to an intradermal dosing regimen of four injections of 6 nmol Cat-PAD every 4 weeks, eight injections of 3 nmol Cat-PAD every 2 weeks, or placebo [12••]. Efficacy was evaluated over four consecutive daily cat allergen challenges in an environmental exposure chamber (EEC). At 50 to 54 weeks post-treatment initiation, only the 6 nmol group demonstrated a strong reduction in mean total rhinoconjunctivitis symptom scores (TRSS, sum of nasal and ocular symptoms) compared to placebo (– 6.778 vs. – 2.908) [12••]. When efficacy was re-evaluated over four cat allergen EEC challenges 2 years after the start of treatment, a trending reduction in mean TRSS was observed in the 6 nmol group over placebo (– 5.87 vs. – 2.02), thus showing the persistent treatment effect of only four injections of Cat-PAD given over 3 months [13••]. However, these findings were not replicated in a larger multicenter, phase III field study of 1245 cat-allergic participants with moderate or severe AR symptoms (mean TRSS ≥ 10) and who lived with a cat at home. Both dosing regimens of four and eight intradermal injections of 6 nmol Cat-PAD performed similarly to placebo, leading to comparable improvements in mean combined scores (sum of TRSS and rescue medication use score) 1 year after treatment initiation (58.2 and 59.8% vs. 58.5%) [14].

Alongside Cat-PAD, seven synthetic peptides were derived from immunodominant T cell epitopes of the major HDM allergen *Dermatophagoides pteronyssinus* and formulated as a 7-peptide vaccine (HDM-PAD). When clinical efficacy was evaluated over three consecutive daily HDM allergen challenges in an EEC, only HDM-allergic participants receiving 4 ×

12 nmol HDM-PAD (four intradermal 12 nmol injections given every 4 weeks) showed a significant reduction in mean TRSS compared to placebo (-5.82 vs. -3.04) 49 to 50 weeks after treatment initiation [15•]. In contrast, participants randomized to 11×12 nmol HDM-PAD showed no improvement in symptoms over placebo, possibly indicating an optimal number of injections required to establish tolerance. In the subsequent 2-year follow-up study, a sustained treatment effect was evident, with the 4×12 nmol group demonstrating a trending reduction in mean TRSS compared to placebo (-6.49 vs. -4.70) [16]. However, similar to Cat-PAD, a larger phase IIb field study of 714 HDM-allergic participants with moderate or severe AR symptoms (mean TRSS ≥ 12) did not meet its primary endpoint of a reduction in mean combined scores compared to placebo after 12 weeks of treatment. Following randomization, the 4×12 , 4×20 , and 8×12 nmol intradermal dosing regimens with HDM-PAD achieved reductions in combined scores comparable to that of placebo (34.9, 40.9, and 44.3% vs. 39.1%), thus mirroring the placebo effect observed in the phase III field study with Cat-PAD [17].

SPIREs have also been constructed for common seasonal allergens including ragweed and several grass pollen species. Ragweed-SPIRE contains several peptides derived from major T cell epitopes of Amb a 1. A preliminary phase II study conducted in an EEC demonstrated a 97% reduction in mean TRSS when compared to placebo (-5.77 vs. -2.93) in a sub-cohort of ragweed-allergic participants with moderate baseline symptomology and who received the highest dose of Ragweed-SPIRE [18••]. In a subsequent phase IIb chamber study, 280 ragweed-allergic participants were randomized to placebo or one of three Ragweed-SPIRE intradermal dosing regimens; 4×6 , 8×6 , and 8×12 nmol [19]. Treatment was completed before the start of ragweed pollen season with efficacy evaluated during post-treatment ragweed pollen challenges in an EEC after the peak of pollen season. The largest dose (8×12 nmol) of Ragweed-SPIRE achieved the greatest reduction in mean TRSS compared to placebo (1.25 point-decrease on top of placebo) [19]. Across both studies, a dose-response effect was evident, with participants randomized to the largest dose of Ragweed-SPIRE experiencing the greatest reduction in allergic symptoms. Contrasting the findings with Cat-PAD and HDM-PAD, higher doses of Ragweed-SPIRE above 8×12 nmol may confer added clinical benefit. However, additional studies are necessary to investigate this possibility.

Another set of SPIREs derived from major grass allergens (Grass-SPIRE) has been recently evaluated in a phase II, dose-finding study of 282 grass-allergic participants conducted in the environmental exposure unit (EEU) [20••]. Using a similar study design as the aforementioned phase IIb EEC study, participants were randomized to one of three dosing regimens: 8×6 nmol (injections every 2 weeks), 8×12 nmol (injections every 2 weeks), and 4×12 nmol (injections every 4 weeks). After the peak of grass pollen season, only the 8×6 nmol group showed a significant improvement in mean TRSS during post-treatment grass pollen challenges in the EEU (42% improvement over placebo) [20••]. Participants were also re-invited to attend an optional follow-up study conducted after the second and third grass pollen season (1 and 2 years after treatment initiation, respectively). After follow-up grass pollen challenges in the EEU, the 8×6 nmol group experienced trending reductions in mean TRSS compared to placebo one (-6.0 , $P = 0.0535$) and two (-6.2 , $P = 0.113$) years after treatment initiation [21•, 22]. Thus, mirroring the long-term treatment effect of Cat-PAD

and HDM-PAD, four intradermal doses of Grass-SPIRE are efficacious in reducing grass-allergic symptoms, with clinical benefit lasting up to 2 years after the first dose.

In clinical practice, the severity and management of asthma are often assessed in evaluating whether patients are eligible for immunotherapy injections. Those with severe or uncontrolled asthma have an increased risk of experiencing asthma exacerbations and life-threatening anaphylaxis during AIT [25]. To date, only two randomized, placebo-controlled studies have evaluated the use of SPIREs in mild asthmatics with safety as the primary outcome. When cat-allergic participants with mild asthma were treated with 8×3 nmol Cat-PAD, reductions in forced expiratory volume in 1 s (FEV_1) $> 30\%$ were not observed across all three cohorts classified according to type asthma medication being taken (salbutamol inhaler, inhaled corticosteroids [ICS], or ICS plus a long-acting β_2 agonist) [26]. A similar study was conducted with HDM-allergic participants with either GINA step 1 (as-needed short-acting β_2 agonist [SABA] with no controller) or 2 (low-dose ICS with as-needed SABA) controlled asthma treated with 4×12 nmol HDM-PAD. After dosing, reductions in FEV_1 or peak expiratory flow rate $> 20\%$ were not observed [27]. Thus, both studies illustrate a favorable safety profile of Cat-PAD and HDM-PAD in allergic patients with controlled asthma.

In both field studies of Cat-PAD and HDM-PAD, remarkable improvements in symptom scores were seen in placebo-treated participants. The placebo effect is frequently noted across AIT trials and represents a barrier in translating positive findings from phase I/II trials to larger phase III field studies. For example, an analysis of five phase III SCIT field studies reported a placebo response of up to 51.8% during the first year of treatment [28]. A possible explanation for the high placebo response can be insufficient allergen exposure during treatment. Both phase II studies for Cat-PAD and HDM-PAD were conducted in controlled allergen challenge facilities, where all participants were challenged to a known concentration of airborne allergen [29•, 30]. In contrast, inadequate exposure to cat or HDM at home (i.e., a field study) can lead to a lack of symptoms, which may create a false perception of treatment benefit in patients assigned to placebo [31]. Patients can also become habituated to their allergic symptoms during treatment and may experience a perceived sense of improvement.

Taken together, current SPIRE studies highlight the possibility of an optimal number of injections and peptide concentrations required to achieve long-term tolerance such that administering larger doses confer no added clinical benefit. For example, 4×6 nmol Cat-PAD, 4×12 nmol HDM-PAD, and 8×6 nmol Grass-SPIRE were all superior to regimens containing additional injections and/or higher peptide concentrations. SPIREs also lack the need for a build-up or up-dosing phase, thus requiring fewer injections to achieve desensitization. During SCIT, the up-dosing phase precedes the maintenance phase and includes injections of increasing allergen doses delivered over several days to weeks depending on the schedule used (i.e., conventional, cluster, or rush) [32]. However, this phase is accompanied by a higher number of systemic adverse reactions in both pediatric and adult populations receiving SCIT [33, 34]. Thus, compared to classical SCIT, some advantages of SPIREs include the improved

safety profile, tolerance towards higher doses, and the requirement of only a few injections given every 2 to 4 weeks.

Allergen tolerance and biological mechanisms of SPIREs

Establishing sustained allergen tolerance is one of the primary goals of AIT. Although allergen can be delivered through several routes (e.g., subcutaneous, sublingual, and intradermal), the acquisition of tolerance is often accompanied by common changes in both cellular and humoral immune responses [35]. A reduction in mast cell and basophil degranulation along with a reduced risk of IgE-mediated anaphylaxis can be seen within the first several hours of initiating AIT. These changes are then followed by the generation of allergen-specific regulatory T (Tregs) and B cell (Bregs) subsets [35]. Tregs play an important role in suppressing allergen-specific effector T cells (e.g., T helper 2 [Th2] cells) [36]. Bregs serve as a source of allergen-specific IgG4 (sIgG4), often described as a blocking antibody due to their ability to inhibit allergen binding to cell-bound sIgE [37, 38]. Both regulatory subsets also produce IL-10, an anti-inflammatory cytokine critical in the maintenance of peripheral tolerance [39–41]. Alongside cellular changes, the level of serum sIgE experiences an initial rise followed by a continuous decrease while serum sIgG4 levels gradually increase over the course of treatment. Finally, long-term decreases in tissue resident mast cells/eosinophils and their pro-inflammatory mediators are often observed several months into AIT [35].

The exact mechanism of peptide immunotherapy is unclear. However, it is thought to share some of the key cellular changes mentioned previously. SPIREs are thought to elicit allergen tolerance by directly binding to MHC class II molecules on APCs along with upregulation of allergen-specific Tregs. Compared to natural allergen extracts, SPIREs are unlikely to elicit robust antibody production due to their short length and lack of B cell epitopes (e.g., IgE epitopes) [10]. To date, mechanistic studies have been conducted with synthetic peptides derived from Fel d 1 and phospholipase A2 (PLA2, major bee venom allergen). When cat-allergic asthmatics were treated with Fel d 1 peptides, proliferative responses were reduced in cat allergen-stimulated peripheral blood mononuclear cell (PBMC), and T cell cultures compared to pre-treatment values [42–44]. IL-13 production from cat allergen-stimulated PBMCs was also lowered after treatment. Similar findings were observed in bee venom-stimulated PBMCs collected after PLA2 peptide treatment, possibly indicating a peptide-induced shift from Th2 cells to Th1 cells and/or Tregs [45]. Providing some support for this theory, Fel d 1 peptide treatment led to increased skin recruitment of CD4⁺ IFN- γ ⁺ T cells and CD4⁺ CD25⁺ T cells 24 h after an intradermal cat allergen challenge compared to diluent challenge and baseline numbers [46•]. Thus, altering the balance between allergen-specific effector and regulatory T cell subsets may be important in peptide-induced tolerance.

When PBMCs from peptide-treated patients were stimulated *ex vivo* with different Fel d 1 peptides, Campbell et al. [47•] noted a reduction in both cell proliferation and Th2 cytokine production against four additional Fel d 1 peptides not included in the original vaccine. This suggests that, within the same allergen, peptides derived from a single epitope can suppress pro-inflammatory responses against other epitopes. This was termed 'linked epitope suppression'

and was shown to be associated with IL-10 using a transgenic mouse model of cat allergen-induced asthma [47•].

More recently, two studies evaluated the effect of SPIRE treatment on immune-associated genes using the nasal allergen challenge (NAC) model, a well-established experimental model of AR [48]. Cat-allergic participants were treated with 4×6 nmol Cat-SPIRE and underwent a NAC pre- and post-treatment to assess efficacy. Whole blood samples were also collected before, 4 weeks, and 24 weeks post-treatment. Four weeks after treatment, several genes related to Th2 cytokine pathways (i.e., IL-3, IL-4, IL-5, IL-6, and thymic stromal lymphopoietin) were down-regulated 6 h post-NAC. These changes were also accompanied by post-treatment reductions in both total nasal symptom scores and peak nasal inspiratory flow [49]. Moreover, Fel d 1-specific T cell expression of IL-8 was significantly reduced after treatment, which correlated with reductions in peripheral blood neutrophils after the NAC [50]. Both studies highlight the effect of SPIREs on the transcriptomic profiles of blood cells, which may contribute to the clinical benefit seen in previous SPIRE trials.

Conclusion

Peptide immunotherapy with SPIREs is a promising alternative to natural allergen extracts and other forms of AIT. These synthetic peptides are derived from major T cell epitopes and lack IgE reactivity, thus reducing the risk of both IgE-mediated immediate adverse reactions and systemic anaphylaxis during treatment. SPIREs also require fewer injections to achieve desensitization compared to classical SCIT dosing regimens.

Current SPIREs have been developed for cat, HDM, ragweed, and grass allergens and are in phase II/III stages of clinical development. Although efficacious in reducing allergic symptoms, the marked placebo effect seen in recent phase III field studies of Cat and HDM-SPIREs represents a challenge in their development. The exact mechanism of action of SPIREs also remains unclear. Studies of both Fel d 1 and PLA2-derived peptides report a shift away from allergen-specific Th2 cells and towards a Th1/Treg phenotype, changes in pro-inflammatory cytokine production, and changes in transcriptomic profiles of immune cells. These changes likely work in tandem to establish sustained allergen tolerance during peptide treatment.

Compliance with ethical standards

Conflict of interests

AKE has participated in advisory boards for ALK Abello, Circassia Ltd., GlaxoSmithKline, Johnson & Johnson, Merck and Novartis, and has been a speaker for Aralez, AstraZeneca, Boehringer Ingelheim, Meda, Mylan, Merck, Novartis, Pediapharm, Pfizer, and Takeda. Her institution has received research grants from Circassia Ltd., Green Cross Pharmaceuticals, GlaxoSmithKline, Sanofi, Sun Pharma, Merck, Novartis and Pfizer. AKE is also a consultant to Bayer Inc. MWT has no relevant conflicts of interest to disclose.

Human and animal rights and informed consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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