

The Role of Synthetic Peptide Immuno-Regulatory Epitope (SPIRE) in the Treatment of Allergic Disease

Mena Soliman, MBChB, MSc¹
Anne K Ellis, MD, MSc, FRCPC^{1,2, *}

Address

¹Allergy Research Unit, Kingston General Hospital, Kingston, ON, Canada

²Division of Allergy and Immunology, Department of Medicine, Queen's University,
Watkins 1D 76 Stuart Street, Kingston, ON, K7L 2V7, Canada
Email: ellisa@queensu.ca

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Opinion statement

Immunotherapy has been used for over a century for the treatment of allergic rhinitis, starting with the administration of whole allergens which are still in use today. Up-titration of allergen concentrations is thought to induce immune-tolerance, reducing symptoms upon exposure of the same allergen in the natural environment. More recently, peptide immunotherapy research aims to reduce sensitization to the allergen while utilizing a much shorter treatment schedule and avoiding the risk of anaphylactic reactions associated with whole allergen administration. Other immunotherapies undergoing clinical trials utilize linear joined peptides of the natural antigen, which are thought to avoid triggering anaphylaxis by selectively joining non-IgE binding allergen proteins. These novel therapies have been investigated using a variety of methods, including at controlled allergen challenge facilities (CACF) and in phase 3 trials in the “real world” setting. Experimental phase 2 trial designs were also used, the nasal allergen challenge (NAC) protocols, involving the direct exposure of the nasal mucosa to the allergen. The mechanism of action of peptide immunotherapies is thought to vary from that of conventional immunotherapies, though more research is needed to understand how desensitization occurs in each case. A shift from CD4+ to CD8+ Th cells is thought to be one way to explain this and is backed by change in cytokine expression in various experimental models.

Introduction

Allergic rhinitis (AR) is an upper respiratory tract inflammatory reaction to an often harmless environmental allergen. Symptoms of runny and congested nasal cavity and sneezing may impact the quality of life of the patients and has been shown to have an effect on sleep and economic productivity [1]. Symptoms can generally be controlled using pharmacotherapy including anti-histamines, orally or intra-nasally, intranasal corticosteroids, or simply by avoiding the allergen if possible. Such measures have a varied degree of effectiveness in symptom control from one patient to the other or when it is not possible to avoid allergen exposure.

Allergen-specific immunotherapy has been in use for over 100 years and involves incremental exposure to the whole allergen protein, to which the patient is allergic. Several routes of exposure have been employed, most commonly as subcutaneous immunotherapy (SCIT), but also as sublingual immunotherapy (SLIT) as tablets or droplets. Newer, experimental modes of IT include intra-lymphatic injections, and most recently in the form of tooth paste (oral mucosal immunotherapy) [2–4, 5••]. Such administration of whole allergen, often over the course of 3 to 5 years, has been shown to have disease modifying effects lasting several years after the discontinuation of treatment. Traditionally, allergists have mixed the formulation from allergen extracts and generated injections with increasing concentrations to be administered until a maintenance dose is reached. Such methods lack standardization and carry the risk of anaphylaxis. The allergen molecule has the ability to bind and cross-link two IgE molecules, causing the degranulation of eosinophils and mast cells. The results of such treatments have a varied degree of effectiveness, partly due to low compliance; one study noted that less than 20% of patients completed the planned treatment regimen [6••].

SLIT has offered an alternative where allergen doses are standardized, particularly if using tablets rather than

sublingual drops. There has been no reported deaths due to anaphylaxis following the use of SLIT, though itching and discomfort in the mouth/throat is commonly reported [6••, 7•]. The convenience of self-administering the tablets following the first one being administered under supervision, is an advantage to this method. Studies comparing efficacy of SLIT and SCIT treatments have reported comparable results in the long term, though in the initial months of treatment SCIT might be more efficacious in reducing symptoms [6••, 7•]. Compliance, however, is an issue with SLIT as well, where patients might find it a burden to administer the tablet daily. Simultaneously administering tablets for 2 allergens has been investigated, demonstrating the safety of sequentially administering SLIT for one allergen for a period of time followed by a second SLIT, reporting mostly throat discomfort with co-administration but otherwise mild to moderately rated adverse events [8•]. Such a regimen is not currently recommended in the product monographs, however.

There is a need for immunotherapy that provides sustained symptom relief, requiring a relatively shorter duration of treatment, while having an improved safety profile. Such treatment should offer a standardized treatment protocol with a low chance of a severe adverse event or anaphylaxis. Administering allergen peptides instead of whole protein is a promising method where the peptides' sequences are shorter and are not able to cross-link two IgE molecules, reducing the possibility of anaphylaxis to a theoretical zero. The peptides offer specific sections or epitopes that are necessary for B-cell or T-cell binding, possibly resulting in immune tolerance [9••, 10••]. Peptide immunotherapies are relatively easier to standardize and purify and are stable in lyophilized form, offering further advantages to using them in a real-world setting. Early trials have demonstrated immunological changes yet were not successful clinically [11].

Peptide Immunotherapy Studies

Early studies of synthetic peptide immunotherapies were carried out in the mid-1990s by Geffer et al. and provided the first evidence of immune tolerance in cat and ragweed allergic patients [11]. The two 27 amino acid long peptides did not offer greater clinical benefit than the available SCIT treatment at the time, while having late onset adverse events [12]. Current

synthetic peptides under-investigation have engineered multiple shorter peptides, initiated by Larché and Kay [12].

The most advanced in development of the peptide immunotherapies is the Cat-PAD (cat-peptide antigen desensitization) (Circassia Ltd., Oxford, UK) which consists of seven peptides of the common cat antigen Fel d1. Several phase 2 trials have investigated this therapy and have indicated the efficacy of using four intradermal doses of 6 nmol of the lyophilized powder in suspension at 4-week intervals. The regimen was tested at a controlled allergen challenge facility (CACF) which utilizes exposing cat-allergic patients to certain concentrations of the allergen in the air while controlling all other aspects of the environment, such as air temperature, humidity, and purification of the air from other allergens [13•, 14•]. The participants were challenged at baseline over four consecutive days and recorded their baseline symptoms, after which treatment started. After a 3-month treatment period, participants' Fel d 1 induced symptoms were re-evaluated at 18 to 22 weeks, and a subgroup at a further 50 to 54 weeks, from the time of the initial CACF challenge and found the 6 nmol treatment regimen to be efficacious [15••]. A 1-year and a 2-year blinded follow-up studies were conducted and indicated maintenance of the efficacy without the need to reinitiate treatment, the 2-year study demonstrated a 2 or 3 point score difference between patients receiving treatment vs placebo at the second and third hours following exposure [15••, 16••, 17].

A dose-response effect was noted when comparing 3 nmol intradermal injections administered eight times at 4-week intervals vs 6 nmol injections administered four times with the same interval. Although the cumulative allergen dose was equal, the 6 nmol arm was found to be more efficacious [16••]. A similar response was not observed in the latest phase 3 trial that compared four vs eight intradermal injections of the 6 nmol immunotherapy [18]. This might suggest that peptide immunotherapy has a threshold for inducing tolerance, beyond which there is no added benefit for increasing the cumulative concentration or number of injections. Such conclusions need longer studies comparing different treatment plans.

An experimental model of AR in humans, the nasal allergen challenge (NAC) model, was tested using Cat-PAD [19, 20•]. Nineteen cat-allergic participants were screened and challenged at baseline using direct spray of specific concentrations of cat allergen into the nasal cavity. Subjective and objective measures of AR were recorded. The patients then received four doses of Cat-PAD 6 nmol over 12 weeks and a second NAC visit was conducted 1 month after the conclusion of treatment [19]. Statistically significant reduction in AR symptoms was observed and improvement in peak nasal inspiratory flow at several time points [19].

AR is usually assessed using subjective measures, mainly the total nasal symptom score (TNSS), composed of nasal congestion, nasal itch, sneezing and rhinorrhea each graded from 0 to 3 for a total out of 12 [21]. The total rhinoconjunctivitis symptom score (TRSS) is also used where participants record their TNSS in addition to ocular pruritus and watery eyes for a score out of 18, or can also include itchy ears/throat/palate and red/burning eyes for a score out of 24 [21].

Recently a phase 3 randomized placebo controlled trial of cat specific peptide immunotherapy (Fel d1) included three treatment groups, 4 × 6 nmol, 8 × 6 nmol (each dose 4 weeks apart), and a placebo group. Participants had to

live with a cat to be included. The primary endpoint was the difference in the mean combined score of TRSS and rescue medication use, between the treatment and placebo groups after 1 year from the start of dosing [18]. Although the two treatment groups (4×6 nmol and 8×6 nmol; each dose 4 weeks apart) reported approximately 58% reduction in symptoms, the placebo group experienced a similar reduction compared to baseline values, causing the study to miss its primary endpoint [18]. Such strong response from the placebo group is not unusual and has been reported in many previous studies. One potential explanation for the high placebo response could also have been that since participants had to live with a cat they could well become 'tolerant' of their background symptoms over time, and less bothered by them, leading to a subjective sense of improvement. A review of five SCIT and one SLIT randomized double-blind placebo controlled trials identified the placebo effect to be up to 41% in the second treatment year for SCIT treatment but was not observed in the SLIT trial [22]. Such effect may dampen the response to treatment in such studies, due to the subjective nature of end points. AR is a subjective disease, in the sense that unless patients are sufficiently annoyed by it they might not seek medical attention. As such, previous trials have relied on subjective measures of AR as well as objective ones [14•, 21].

Apart from development in cat-allergen immunotherapy, several other immunotherapies are at different phases, including for house dust mites, ragweed, and grass [23, 24]. As with Cat-PAD, seven synthetic house dust mite peptides were selected using in vitro analysis of their ability to function as epitopes. A phase 2 safety study concluded the tolerability of these peptides and a further phase 2b study evaluated its efficacy after 1 year from starting dose administration [23, 25••]. The study followed a similar course to the Cat-PAD studies where intradermal injections were administered at 4-week intervals; however, it compared administering 4 vs 11 injections in a randomized double-blinded placebo controlled trial [25••]. In a similar dose-response manner to that observed with cat allergen, administering 11 injections did not achieve better symptom control compared to four. The four injections of 12 nmol provided positive results at the end of the study, achieving TNSS reduction of 5.8 compared to baseline while the placebo group reported a decline of 3.0 by week 50 of the CACF study [25••]. Of note is the safety profile of all the different allergens were comparable to placebo with no reported systemic reactions.

A multicenter, randomized, double-blinded, controlled, parallel group study of grass SPIRE at the Environmental Exposure Unit (EEU, Kingston, ON, Canada) conducted in 2012 demonstrated the efficacy of treatment over 14 weeks [26]. An optional double-blinded follow-up study was performed following exposure to two grass pollen seasons, where the original recipients of treatment were invited to participate in EEU allergen challenge visits [27•]. The treatment arm experienced a mean TRSS reduction of 6 vs 3.6 for the placebo arm ($p = 0.535$). Similar to Cat-PAD, the 6 nmol treatment was found to be more effective than the 12 nmol group [27•].

Though they are not made of synthetic peptides, a group of immunotherapies are being investigated by Biomay AG (Vienna, Austria) that are made from fused linear peptides from the natural antigen [28, 29]. The non-IgE binding portions of the protein and fused to a viral protein and is used as immunotherapy. The immunotherapy is thought to stimulate B-cells to produce allergen-

specific IgG that will bind to the natural allergen upon exposure and act as a blocking antibody, preventing cross-linking of IgE and mast cell degranulation [30]. By joining the peptides to a viral protein, the vaccine is thought to retain its capability of stimulating the T-cell response as well [30].

Mechanism of Action

The mechanism of action of whole allergen immunotherapy is not well understood with some conflicting reports around how tolerance evolves to the allergen of interest. The route of administration of the allergen can also add to the variability in the mechanism of action, particularly between SCIT and SLIT. Peptide immunotherapy is thought to share some mechanistic attributes with SCIT in terms of induction of tolerance; both cellular and humoral immune responses have been observed though weaker with peptide immunotherapy.

Observation of changes in T-cell populations have shown a shift from T-helper-2 cells (Th2 or CD4+) to T-helper-1 cell response (Th1, CD4+ IFN- γ +) [31, 32]. Such changes were also associated in a reduction in the concentrations of pro-inflammatory cytokine response, such as IL-5 and IL-13, which are also promoters of inflammatory cell migration to the local mucosal tissue [32]. As a result of this shift away from pro-inflammation, less eosinophils, basophils, and mast cells have been noted. Although there is a decrease in pro-inflammatory cells, repeated skin prick testing is not recommended as a measure of success of therapy [33]. Subjective assessment of symptoms of AR and allergic conjunctivitis are better suited, though might be affected by a strong placebo-like effect [33].

Others have reported an increase in IL-10 and increased recruitment of CD4+ CD25+ T-regulatory cells, a possible explanation of the shift away from pro-inflammatory state [32, 34, 35, 36••]. Blocking of IL-10 receptors in a mouse model was shown to reverse treatment effect on recruitment of IL-10+ T-cells associated with immune tolerance [34]. Alexander et al. have suggested that an increase in tissue growth factor— β (TGF- β) might also be an indicator of induction of tolerance [36••]. The detection of such changes in SCIT is usually associated with long-term decrease in allergen-specific T-cell response. On the contrary to these findings, Smith et al. have noted no such changes to naturally occurring regulatory T-cells CD4+/CD25+ in peripheral blood, challenging the original theory [31]. A recent analysis of FoxP3 in Tregs has shown increased staining intensity per cell, suggesting increased regulatory transcription factor per cell [37]. This might help explain Smith et al.'s observations.

Campbell et al. have observed that treatment with selected epitopes from a single allergen was capable of suppressing responses to other epitopes within the same molecule, suggesting that response to epitopes of the same allergen are linked [34]. The use of a selected group of epitopes of the allergen might be sufficient to induce tolerance to the whole allergen.

One consistent finding from multiple experiments is the weak humoral response, possibly due to absence of confirmation B-cell epitopes on short peptides. Though there is a weak B-cell response, subsequent exposure to the natural occurring antigen can lead to increased production of IgG4, such as that observed with bee peptide immunotherapy [38••]. IgG has been suggested to

play a role as a “blocking” antibody, thought to bind to the allergen, preventing the cross-linking of sIgE on eosinophils and mast cells [11, 39]. There is no evidence to suggest a correlation between increased IgG4 levels and clinical improvement, and is not predictive of the degree or duration of efficacy of the immunotherapy [33, 40].

Conclusion

Peptide immunotherapy remains a promising potential treatment option, offering reduced treatment schedules with far less risk of severe adverse effects. Such advantages are driving further development and interest in this field. Relatively new peptide immunotherapies offer shorter, more convenient treatment schedules while reducing the risk of anaphylaxis compared to more conventional whole allergen therapies. Such novel therapies are being tested using a variety of clinical protocols in phase 2 and 3 studies, including controlled allergen challenge facilities (CACF) and nasal allergen challenge (NAC) models. Advances in the mechanism of action of these peptides might help in future peptide epitope selection and trial designs, with advances being made using human and animal models. Different mechanisms of action have been suggested including shift from CD4+ to CD8+ Th cell expression, to increased Treg expression, and some studies seem to suggest B-cell role in certain immunotherapies. More research is needed to fully evaluate each of the novel therapies and further understand their mechanism of action.

Compliance with Ethical Standards

Conflict of Interest

Mena Soliman declares no conflicts of interest. Dr. Anne K Ellis declares grants to institution from Novartis, Pfizer, and GSK; Speaker’s Bureau from Novartis, Pfizer, Takeda, Meda, and Astra Zeneca; and is an Advisory Board Member for Novartis, Circassia Ltd and GSK.

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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