



Immunoregulatory T cell epitope peptides for the treatment of allergic disease

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Allergic diseases are type 2 inflammatory reactions with an increasing worldwide prevalence, making the search for new therapeutic options pertinent. Allergen immunotherapy is the only disease-modifying approach for allergic rhinitis, though it can result in systemic reactions. Recently, peptide immunotherapy (PIT), involving T cell epitope peptides that bind to major histocompatibility complexes, have been developed. It is speculated that they can induce T helper cell type 2 anergy, Treg cell upregulation or immune deviation. Promising results in cat dander, honeybee venom, Japanese cedar pollen, grass pollens, ragweed and house dust mite clinical trials have shown safety, efficacy and tolerability to PIT. Hence, PIT may hold the potential to change the treatment algorithm for allergic rhinitis.

Lay abstract: Allergen immunotherapy is the only disease-modifying treatment option for patients with chronic allergic rhinitis, typically featuring formulations to promote the development of immune tolerance. Although effective, the use of whole allergen has been found to promote unintended IgE crosslinking, resulting in local and systemic adverse effects. To overcome such limitations, the use of T cell epitope peptides in allergen immunotherapy has been explored with hopeful outcomes. Compared with allergen immunotherapy, peptide immunotherapy is quicker, safer and more efficacious, making it quite promising. Although peptides for pet dander, honeybee venom, pollen and house dust mite allergies have been developed, more extensive research is necessary to prior to widespread usage.

Tweetable abstract: Clinical trials have shown beneficial safety, efficacy and tolerance with T-cell #peptide #immunotherapy. It shows great promise as upcoming treatment for allergic rhinitis.

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Allergic diseases

Allergic diseases are a T helper (Th)2-inflammatory reaction by IgE. In general, the mechanism of action involves sensitization, wherein the exposed allergen is inhaled and presented on dendritic cells, an early phase reaction, characterized by an immediate release of pro-inflammatory mediators and a late-phase reaction involving the recruitment and degranulation of various leukocytes.

The prevalence of allergic disease continues to increase globally. Among adolescents, allergic diseases are the most prevalent chronic condition. Major manifestations include asthma, allergic rhinitis (AR), conjunctivitis, anaphylaxis, eczema, urticaria and angioedema; all of which negatively impact one's quality of life [1]. AR is characterized by excessive sneezing, rhinorrhea and nasal congestion due to local inflammation of the nasal mucosa. AR is often seen in combination with conjunctivitis causing swelling and irritation of the eye, which can severely impair one's performance in school or work settings [1]. For this reason, identifying appropriate treatments for allergic diseases, and AR in particular, has become increasingly important.

Allergen immunotherapy

Although pharmacotherapeutic treatments, such as antihistamines and nasal corticosteroids, help reduce the allergen-induced symptoms of AR, their effects are transient and not curative. Allergen immunotherapy (AIT) is the only disease-modifying treatment option. AIT consists of an 'up-dosing' phase, where increasing doses of whole or fragmented allergen extracts are delivered subcutaneously or sublingually, followed by the 'maintenance' phase, wherein a steady allergen-extract dose is administered spanning from months to years [2]. The end goal is to induce immunological and clinical tolerance for a particular allergen [3,4]. AIT typically employs the use of whole allergen, which tends to crosslink IgE, hence promoting the unintended activation of mast cells and basophils, resulting in adverse local and systemic reactions including anaphylaxis [5,6].

Peptide immunotherapy

Fortunately, thymus-maturing lymphocytes (T cell) epitope peptide immunotherapy (PIT) provides a promising direction to overcome the disadvantages of AIT, while simultaneously reducing the burden of allergic disease [7]. PIT involves the administration of short synthetic peptide immunoregulatory epitope (SPIRE) derived from a particular allergen. It avoids IgE-mediated leukocyte activation and degranulation while still allowing the quiescent presentation of allergen-derived T cell epitopes to antigen-presenting cells. Ultimately, this promotes an anti-inflammatory response when exposed to allergens, in attempt to induce anergy [8]. These synthetic peptides can be produced at a low cost, with eased purification methods and appropriate stability in its lyophilized form, thereby eliminating the need for cold storage [9]. Although T-cell epitope PIT may stimulate T-cell tolerance, its high safety profile compared with AIT makes it a promising disease-modifying treatment. In fact, contrary to AIT, large concentrations of PIT can be administered in shorter treatment periods, demonstrating significant improvements within 2 years of the onset of treatment [10]. The shortened treatment timeframe of PIT is beneficial as patient compliance for the burdensome AIT regimen is decreasing, as a study by Kiel *et al.* found that less than 20% of AIT patients completed the full 3-year treatment period [11]. Generally, PIT offers the benefits of AIT with reduced side effects.

Mechanism of action

When administered, T cell epitope peptides attach to the major histocompatibility complex (MHC) class II on antigen-presenting cells, after which they can bind to T cell receptors [3]. PIT's exact mechanism remains unclear; however, clinical data speculates this may include the induction of Th2 anergy, upregulation of Treg cell responses and a combination of immune deviation and induction of Treg cells [12–14].

To begin, results of some clinical trials suggest that PIT induces Th2 cell anergy. Cytokine analysis by Muller *et al.* studying bee venom PIT using peptides from phospholipase A2 revealed a decrease in IL-2, IL-4, IL-5, IL-13 and IFN- γ as well as phospholipase A2-induced proliferative responses in peripheral blood mononuclear cell levels [9]. Similarly, a reduction in Th2 cytokines, unchanged allergen-specific IgE, and preferred IgG4 levels were observed post allergen exposure, supporting the anti-inflammatory and tolerance-inducing effects due to PIT [15]. It has been suggested that this anergy is achieved by Th2 cell deletion. Wambre *et al.* used human leukocyte antigen class II tetramers to track clonal T cell populations for Timothy grass pollen. Results showed a preferential deletion in specific Th2 cells upon administration of dominant Timothy grass pollen epitopes, while the abundance of Th1 and type 1 Tregs (Tr-1) remained unchanged. Results were reinforced as cytokine analysis revealed a decrease in the level of cluster of differentiation -27 (CD27) expression [12].

PIT may also upregulate Treg cells, whose tolerance failure gives rise to allergic diseases [13]. Alexander *et al.* observed a minimal increase in Treg cell recruitment post administration of Fel d 1-derived peptides [16]. Nonetheless, these results were inconclusive and somewhat counter intuitive, as an increase in transforming growth factor- β , detected by analyzing mRNA expression, was observed in the absence of IL-10+ [17,18].

The most widely accepted mechanism hypothesizes that PIT induces a Treg cell-favored mechanism, as opposed to a traditional Th2-mediated mechanism (Treg) [19]. In this process, evidence supports the indispensable role of IL-10, as a key immunosuppressor, as it has been repeatedly observed alongside post-PIT during subsequent allergen challenges [20–22]. Furthermore, a comparative study by Royer *et al.* hypothesized that the increase in IL-10 secretion from Treg cells is due to an increased expression of SOCS1 molecules [23]. Interestingly, due to an increased IL-10 production enhances the IL-4 induced isotype switching of IgG4, which has suggested roles as a 'blocking antibody' to prevent allergen crosslinking, a common and unavoidable problem in conventional AIT [14,24]. Overall,

the preferential switch to a Treg response induces development of allergen tolerance, shortening the duration of an allergic reaction and preventing propagation to more severe allergic manifestations [25].

Development & selection of suitable peptides

In general, T cell epitope peptides are selected based on immunogenicity, the ability to stimulate either CD4 or CD8 cells. Those designed for MHCI are restricted in length given the closed peptide-binding cleft of the complex. Thus, these peptides typically range between nine and 11 amino acids long joined by hydrogen bonds. Conversely, the open groves of MHCII allow longer epitope peptides to be administered [26]. Initially, the epitope peptide sequencing algorithm was based on sequence motifs, amino acid preferences at specific MHC anchor positions [27]. This was quickly replaced by motif matrices as nonanchoring residues also contribute to MHC binding. Unlike sequence motifs, motif matrices evaluate every possible peptide-binding position, yielding accurate readouts of peptide–complex interaction [28,29]. A similar approach called quantitative affinity matrices is coupled with peptide-binding affinity values for a more accurate measure of epitope efficacy [30,31].

There are numerous ways in which T cell epitopes can be identified and characterized. The most frequently used methods include the generation of T cell lines, isolation of endogenous ligands and use of bioinformatic tools, such as mimotope libraries, to predict human leukocyte antigen class II binding propensity [32]. For simplicity purposes, all such epitope peptide identification techniques can be broadly categorized as either structure-based or data methods.

Structure methods include using an online simulation to map and visualize the epitope peptide and MHCII complex [33]. Conveniently, continuous experimental data is not needed for structural identification. Unfortunately, this results in less effective peptide performance post administration, primarily due to an unpredictable solubility [34,35]. Conversely, data-based methods include using identified MHCII-binding peptide sequences found in databases, such as Immune Epitope Database and Analysis Resource [36], Epitope Peptides in MHC [37] and AntiJen [38]. Irrespective of using structural or data-based methods employed for identification of peptides, their reactivity is tested using a competitive ELISA for allergen-specific IgE, after identifying potential epitope peptides. Often several peptides are tested to ensure the greatest binding and peripheral blood mononuclear cell reactivity [39–41]. Selected epitopes are subsequently prescreened using population cohorts, wherein they are administered to those sensitive to a common allergen prior to clinical implementation [42]. Epitope recognition techniques, both computer and simulation based have made such progress that 1406 CD4+ T cell epitopes have been derived from human T cells [43]. Interestingly, machine learning, specifically the models of artificial neural networks, support vector machines, decision trees and Hidden Markov models provide a promising direction toward integrating structure and data-based methods to yield optimal, cost-effective and more stable and soluble synthesized peptides [44].

Allergen-specific peptide immunotherapies

Preclinical models have established the feasibility of the use of T cell peptides for immunotherapy (Figure 1). Here, clinical results reporting the safety, efficacy and tolerability of PIT for treating AR are explored.

Pet dander allergy

Pet dander allergies mainly represent reactions to the skin, saliva or urine of cats and dogs. In the scope of PIT, T cell peptides for Fel d 1, theP major cat allergen, have been studied extensively. Two synthetic peptides of 27 amino acid in length, IPC-1 and IPC-2 (ALLERVAX CAT), were developed containing major T cell epitopes and demonstrated minimal binding to Fel d 1-specific IgE [45,46]. In a safety trial, three doses of ALLERVAX CAT (7.5, 75 and 750 µg) were compared with placebo [47]. Participants were exposed to live cats before and after immunotherapy treatment, while being asked to report their lung and nasal symptom severity throughout treatment. After 6 weeks of treatment, researchers found that the 750 µg dose had the greatest effect, while no significant difference was seen with 7.5 µg of ALLERVAX CAT. Subsequently, a randomized, multicenter, double-blind trial involving 133 cat-allergic participants evaluated two concentrations of ALLERVAX CAT (75 and 750 µg) to placebo [48]. The higher dosage was effective at improving pulmonary function compared with the placebo and was well-tolerated by participants. Interestingly, all participants had serum IgE against the whole cat antigen, though few had detectable levels of antibodies to ALLERVAX CAT. The most common adverse events, seen to decrease with successive treatment injections, included chest tightness, coughing, wheezing or throat irritation. Despite side effects, researchers observed improved tolerance to cat dander as a result of PIT. In another 4-week study, Simons *et al.* found that a 250 µg dose of T-cell PIT did not improve skin reactivity to cat allergen extract

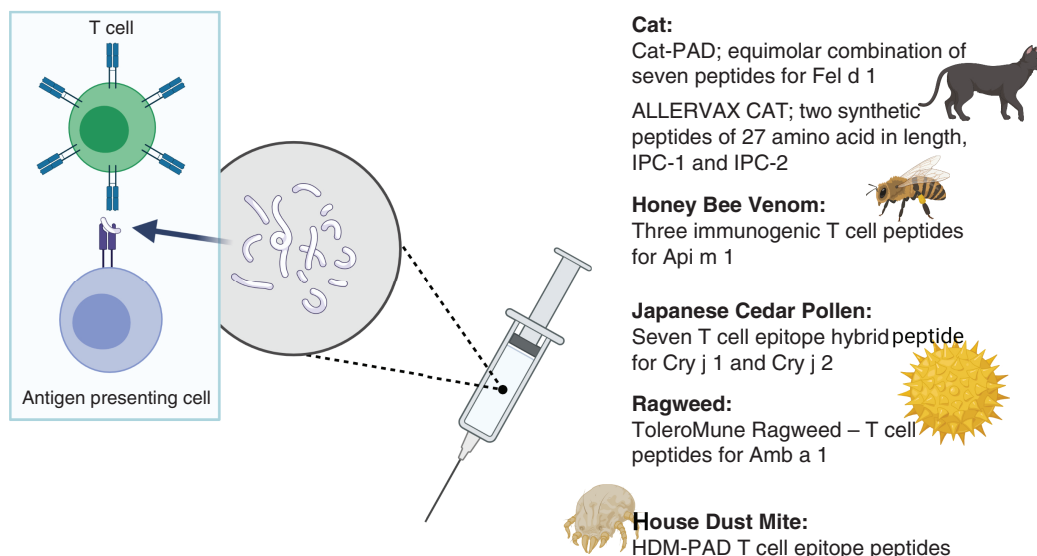


Figure 1. The different allergen-specific peptide immunotherapies.
ALLERVAX CAT; HDM: House dust mite; PAD: Peptide antigen desensitization.

compared with baseline. However, they observed delayed onset of AR symptoms following injection treatment compared with placebo [49].

Further studies have investigated a peptide vaccine featuring an equimolar combination of seven peptides from Fel d 1, termed Cat Peptide Antigen Desensitization (Cat-PAD). Worm *et al.* generated a vaccine and evaluated the safety and tolerability of both intradermal and subcutaneous routes of administration for these immunotherapy injections with doses ranging from 0.03 to 12 nmol. Of the 88 eligible participants, 40 received intradermal injections of either the peptide (75%) or placebo (25%) while 48 were given subcutaneous injections (36 received treatment and 12 were given placebo) [50]. No serious adverse events were reported; however, the intradermal treatment group reported side effects of nasopharyngitis, cough and headache, while the subcutaneous treatment group reported experiencing various respiratory symptoms. Intradermal injections resulted in transient local reactions at the injection site for all participants; however, this was not observed following subcutaneous administration. Cytokine responses to the peptides were weak or moderate; however, the combination of the seven peptides in the vaccine produced a biological response equivalent to that with cat allergen extract. A 3 nmol intradermal injection of Cat-PAD, compared with 1 and 12 nmols, was observed to produce the greatest reduction in late-phase skin reactions [50]. Another investigation found that four 6 nmol administration of Cat-PAD given every 4 weeks had apparent and sustained treatment effects for up to a year, compared with eight 3 nmol doses given every 2 weeks and to placebo [51]. Using flow cytometry, Rudulier *et al.* determined that Cat-PAD immunotherapy resulted in a reduction of the chemokine receptor CRTh2 on the cell surface of T cells, though the frequency and phenotypes of Fel d 1CD⁺ T cells were not impacted [52].

To further the development of T-cell PIT for cat allergy, allergen exposure facilities have been employed. A study using the Environmental Exposure Chamber (EEC) found that Cat-PAD was safe, well-tolerated and improved total rhinoconjunctivitis symptom scores (TRSS). The 12–14 week dosing schedule showed greater efficacy than the 6-week treatment period [53]. Furthermore, a randomized, double-blind, placebo-controlled study observed sustained reductions in rhinoconjunctivitis symptoms following allergen challenge in the EEC 2 years after the onset of four administrations of 6 nmol Cat-PAD [54]. This suggests long-lasting therapeutic effects with T-cell PIT. Moreover, a study by Neighbour *et al.* found a significant reduction in total nasal symptom score following four injections of Cat-PAD when using the nasal allergen challenge model of AR [55]. Despite successful Phase I trials, the Phase III Cat-PAD trials failed due to high placebo effects (58.5%) [56]. Of note, high placebo effects in Phase III AIT trials are not uncommon, as total nasal symptom score, often the primary outcome evaluated, is based on participant perception, which is subjective. Other outcomes, such as rescue medication use, may be a better measure of treatment efficacy. Nonetheless, Cat-PAD has shown promise in the treatment of perennial AR with good safety, tolerability and efficacy in clinical trials assessing both short-term and long-term exposure models.

Given that encouraging results have been observed in T-cell PIT for cat dander, dog allergen peptides are becoming an upcoming field of study. Immonen *et al.* have evaluated immune characteristics of epitopes for Can f 1 while Ronka *et al.* have characterized CD4+ T-cell responses to Can f 4. The mapping of several epitopes that may be potential targets for future immunotherapy investigations [57,58].

Honey bee venom allergy

The major allergen in honeybee venom allergy is Api m 1. Researchers established three immunogenic T cell peptides for Api m 1 and investigated its clinical feasibility for immunotherapy [15,59]. Five bee-allergic participants were subcutaneously administered a mixture of the three peptides in increasing doses up to 100 µg for 2 months, and ten participants were treated with standard bee venom immunotherapy as control. Participants administered PIT experienced no allergic side effects due to the immunotherapy treatment and successfully tolerated challenge with 10 µg of whole bee venom allergen. With a live bee sting challenge, three of the five participants experienced no systemic allergic reactions [15]. Subsequently, researchers assessed the potential of a four-peptide specific immunotherapy, developed using peptide–MHC binding assays [60,61]. Twelve bee-allergic participants received nine immunotherapy injections intradermally while six volunteers were assigned to the control group. Post-PIT, significantly decreased late-phase cutaneous reactions and eosinophil recruitment was observed [61]. Fellrath *et al.* completed a Phase I trial to investigate the safety and immunogenicity of a mixture of three long synthetic peptides for bee-allergy immunotherapy. Sixteen participants received five increasing doses of immunotherapy to reach a maintenance dose of 100 µg. Researchers observed the occurrence of T-cell tolerance and decreased cytokine production. No serious, life-threatening adverse reactions occurred [62]. Similar to cat allergy therapies, studies investigating bee venom PIT have shown promising findings.

Pollen allergies

T cell epitopes for seasonal allergens, including grasses and other pollens, have also been investigated. In a preclinical evaluation, researchers designed a seven T cell epitope hybrid peptide for Cry j 1 and Cry j 2, two Japanese cedar pollen allergens out of the many that have showed positive potential in PIT [63]. In a randomized, double-blind, placebo-controlled study using the Environmental Exposure Unit, researchers investigated the safety, efficacy and tolerability of grass allergen T cell peptides. Participants received either a placebo or one of three treatment schedules – eight 6 nmol doses every 2 weeks ($8 \times 6Q2W$), four 12 nmol doses every 4 weeks ($4 \times 12Q4W$) or eight 12 nmol doses every 2 weeks ($8 \times 12Q2W$). Compared with placebo at baseline, a good safety profile and significantly greater reductions in TRSS were observed with the $8 \times 6Q2W$ group [64]. Follow-up studies showed sustained reductions of allergy symptoms at 1 and 2 years following treatment [65,66]. Using the EEC, Hafner *et al.* assessed the safety and efficacy of an immunotherapy vaccine comprised of T cell epitopes generated from the ragweed allergen Amb a 1 (ToleroMune Ragweed). The highest dose of ToleroMune Ragweed produced the greatest change in TRSS from baseline and was well-tolerated by participants [67].

House dust mite allergy

House dust mite (HDM) PAD was also observed to be safe and well-tolerated, with no serious adverse events. In a study by Larche *et al.*, participants were given four intradermal injections of HDM T cell epitopes every 4 weeks. Early and late-phase skin responses were reduced post-PIT [68]. Another investigation with the EEC reported persistent reductions in allergy symptoms 1 year following the induction of HDM-PAD [69]. A follow-up study reevaluated 72 of the 116 participants and found that the 12 nmol dose was associated with sustained symptom reduction at 2 years following treatment [70]. Researchers did, however, report a high placebo effect. A randomized, double-blind, placebo-controlled study evaluated the efficacy of three treatment regimens over 12 weeks: four 12 nmol doses, four 20 nmol doses and eight 12 nmol doses of HDM-PAD [71]. Nonetheless, similar to before, despite improvements in TRSS and rescue medication use, a substantial placebo effect was reported [71].

Conclusion

In summary, compared with traditional AIT, T-cell PIT reduces a patient's IgE-mediated responses while simultaneously improving the burden of disease. Clinical investigations involving T-cell PIT catered for cat, dog, grasses, pollens and HDM allergens have demonstrated good overall safety, tolerability and efficacy with limited adverse events. Nevertheless, high placebo effects have been reported, ultimately warranting further research prior to clinical

implementation. As of now, preliminary data shows encouraging outcomes for T-cell PIT, which may become a pivotal long-term option for AR.

Future perspective

Within the next decade, T-cell PIT is predicted to become more widespread, following further assessments of its safety and efficacy. Comparative studies will also give insights into the effectiveness of T-cell immunotherapy in relation to pre-existing pharmacologic therapies, differing routes of administration and administration period. In the future, there is a positive outlook into the development of a greater variety of peptides of better specificities and greater clinical efficacies.

Executive summary

Allergic diseases

- The prevalence and burden of allergic diseases have risen worldwide.

Peptide immunotherapy

- Peptide immunotherapy (PIT) is a newer therapeutic option, involving the use of T cell epitope peptides rather than allergen extracts, in efforts to reduce systemic IgE-mediated adverse responses, traditionally observed during allergen immunotherapy.
- Although the exact mechanism of PIT is not known, clinical data suggests this may induce T helper cell type 2 (Th2) anergy, upregulate Treg cell responses and/or have a combined mechanism involving a shift from a Th2-mediated to a Treg-mediated response.

Development & selection of suitable peptides

- Epitope peptide selection can be broadly classified as being either structure or data-centered methods, involving online major histocompatibility complex: epitope interaction simulations and database searches, respectively.

Allergen-specific peptide immunotherapies

- Clinical data investigating cat, dog, Japanese cedar pollen, grass pollens, ragweed and house dust mite allergen peptides for PIT have shown good efficacy, tolerability and safety despite reports of high placebo effects.

Financial & competing interests disclosure

AK Ellis has participated in advisory boards for ALK Abello, AstraZeneca, Aralez, Bausch Health, LEO Pharma, Merck, Novartis and Pfizer, and has been a speaker for ALK Abello, The ACADEMY, Aralez, AstraZeneca, Medexus and Mylan. Her institution has received research grants from ALK Abello, Aralez, AstraZeneca, Bayer LLC, Medexus, Novartis, Sanofi and Regeneron. She has also served as an independent consultant to Bayer LLC and Regeneron. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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