

# Peptide Immunotherapy

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Specific allergen immunotherapy is clinically effective and has an extended duration of action following cessation of treatment [1]; however, the therapeutic use of proteins to which the patient is sensitized results in frequent allergic adverse events, most commonly related to cross-linking of allergen-specific IgE on mast cells and basophils. Several strategies aimed at reducing the allergenicity of the treatment preparation while maintaining immunogenicity have been described. Many are discussed elsewhere in this issue. The use of short soluble synthetic peptides for the treatment of allergic disease offers the potential to avoid IgE-mediated allergic reactions while targeting T-cell epitopes and inducing modulation of allergen-specific responses. Synthetic peptides have been evaluated in several experimental animal models and more recently in human studies. Peptides are inexpensive to produce and easy to purify, standardize, and store. Short peptides lack the tertiary structure of the native protein but retain the ability to stimulate T cells.

## **In vitro and in vivo experimental models**

The concept of therapeutic, antigen-specific targeting of T cells for the prevention and treatment of immunologic diseases developed from in vitro studies documenting the effects of high-dose peptide presentation between T cells. CD4+ helper T-cell clones were rendered refractory to antigen stimulation following pretreatment with supraoptimal concentrations of specific peptide [2]. Because antigen-presenting cells were absent, presentation of peptide to T-cell receptors

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most likely occurred through binding to major histocompatibility complex (MHC) class II molecules on T cells and recognition of antigen in a “nonprofessional” context. Subsequent studies have confirmed the ability of nonprofessional antigen-presenting cells to induce T-cell tolerance [3].

Peptide-induced, antigen-specific nonresponsiveness, also known as tolerance, has been demonstrated in numerous murine models of autoimmunity and allergy. Initial experiments demonstrated that it was possible to induce systemic tolerance to peptides administered in incomplete Freund’s adjuvant during the neonatal period [4,5]. Several studies have focused on the prevention and treatment of experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis. High-dose intravenous peptide therapy resulted in T-cell clonal deletion and protection against disease [6]. EAE was also prevented by intraperitoneal administration of peptides from myelin basic protein (MBP). Peptide therapy blocked the progression of disease and decreased severity [7]. Intranasal, but not oral, administration of an MBP peptide led to protection from EAE [8]. More recently in a T-cell receptor transgenic model of EAE, MBP peptides were administered intranasally, leading to protection from disease that was dependent on deletion of effector T cells and the presence of interleukin-10 (IL-10) [9,10].

Peptide treatment has also been evaluated in murine models of experimental arthritis. Neonatal and adult rats were protected from collagen-induced arthritis by administration of peptides from type II collagen [11]. In a separate study, 50  $\mu\text{g}$  of an immunodominant T-cell epitope of type II collagen was given intranasally for 4 days before induction of collagen-induced arthritis. Peptide treatment delayed the onset of disease and reduced severity. The treatment effect was associated with induction of IgG1 antibodies rather than IgG2b [12]. Prakken and colleagues [13] administered a peptide from heat shock protein (hsp60) to rats either intranasally ( $3 \times 100 \mu\text{g}$ ) or subcutaneously ( $2 \times 300 \mu\text{g}$ ). Both treatments protected the animals from the development of adjuvant arthritis induced by heat-killed mycobacterium tuberculosis.

Nonobese diabetic (NOD) mice were protected from diabetes by subcutaneous administration of 100  $\mu\text{g}$  of a peptide of the insulin B chain in adjuvant or intranasal peptide (240  $\mu\text{g}$  total peptide dose) [14]. Bockova and colleagues [15] used two hsp60 peptides to prevent the development of diabetes in NOD mice following subcutaneous administration in adjuvant. Glutamate decarboxylase (GAD65) peptides given intranasally reduced GAD-specific interferon- $\gamma$  (IFN- $\gamma$ ) responses, increased IL-5 responses, and protected mice from disease. CD4+ cells from treated mice blocked adoptive transfer of disease to NOD *scid/scid* mice [16].

Fewer in vivo studies of peptide therapy have been performed in allergen sensitization models. Briner and colleagues [17] sensitized mice intraperitoneally with the major cat allergen Fel d 1. Treatment of mice with two allergen-derived polypeptides resulted in decreased production of IL-2 and allergen-specific IgG. Hoyne and colleagues [18] primed mice intraperitoneally with the major house dust mite allergen Der p 2 and then administered Der p 2 peptides intranasally. Peptide therapy downregulated T-cell and antibody responses to the native pro-

tein. In a model of tree pollen allergy, the dominant T-cell epitope of the major birch pollen allergen Bet v 1 was administered to CBA/J mice prophylactically and therapeutically. T-cell proliferative responses were inhibited by both treatments, but no change in antibody production or isotype was observed [19]. In a murine model of insect venom sensitivity, prophylactic administration of peptides from the bee venom allergen Api m 4 or the hornet venom allergen Dol m 5 resulted in a partial reduction of T-cell proliferation and B-cell antibody responses to subsequent allergen sensitization [20]. In separate studies of insect venom allergy, mice were sensitized with 100 ng of the major bee venom allergen Api m 1 (phospholipase A<sub>2</sub> [PLA<sub>2</sub>]) in alum and treated by administration of 600 µg of a mixture of three polypeptides spanning the entire molecule. Mice were protected from anaphylaxis. A significant reduction in specific IgE was observed together with an increase in allergen-specific IgG2a and a reduced Th2:Th1 ratio [21].

## Clinical studies

### *Autoimmunity*

Peptide immunotherapy has been evaluated in small clinical studies with varying degrees of success. Most studies have focused on individuals with allergic disease, but some have also been performed in autoimmune disease including type I diabetes, rheumatoid arthritis, and multiple sclerosis. In many autoimmune diseases, it remains unclear which antigens should be selected as targets for intervention. Multiple sclerosis studies have focused on peptides from MBP, whereas two recent studies have evaluated peptides derived from heat shock proteins for the treatment of diabetes and rheumatoid arthritis. Patients with newly diagnosed type I diabetes were treated with 1 mg of peptide by subcutaneous injection at three time points (at study entry, 1 month, and 6 months). The treatment peptide (p277) was derived (and slightly modified) from the heat shock protein hsp60 and was selected on the basis of data obtained in experimental animals. Ten months after treatment, islet cell function had declined in the placebo-treated control group but had been maintained in the peptide-treated group. Peptide treatment was associated with modulation of peripheral blood responses to antigen. T cells produced more IL-13 and IL-10 following culture with antigen, indicating modulation toward a Th2/regulatory phenotype [22].

In patients with rheumatoid arthritis, another peptide (dnaJP1) derived from a heat shock protein of bacteria (dnaJ) was administered orally. Patients received 0.25, 2.5, or 25 mg of peptide daily for 6 months. The numbers of antigen-specific cells did not change following peptide treatment, but there were significant increases in the percentage of T cells producing IL-4 and IL-10 and concomitant reductions in T-cell proliferation, IL-2, IFN- $\gamma$ , and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) production [23]. Furthermore, expression of *foxp3* was increased in CD4<sup>+</sup> CD25<sup>bright</sup> T cells. These findings suggest that a regulatory

T-cell response may have been induced by peptide therapy. Clinical data were not reported in this open study.

Patients with multiple sclerosis were treated with a single modified peptide (altered peptide ligand) from MBP in two related studies [24,25]. Patients were treated with up to 50 mg of peptide weekly by subcutaneous injection. The clinical response to peptide treatment was variable, and no definitive improvement in clinical outcomes was observed. Both studies were suspended as a result of treatment-related adverse events. In one study, adverse events were associated with the development of Th2 cells during treatment. Similar reactions in the other study were associated with expansion of peptide-specific Th1 cells displaying increased avidity for peptide-MHC complexes. Clinical deterioration correlated with expansion of antigen-specific Th1 cells during treatment. Subjects treated with lower doses of peptide (5 mg) weekly showed a trend for a reduction compared with baseline in the number and volume of contrast-enhancing lesions following 4 months of treatment.

### *Cat allergy*

The majority of peptide immunotherapy studies in subjects with allergic disease have been performed with peptides from the major allergen of cat dander Fel d 1. Although cat allergy is not as prevalent as house dust mite or grass pollen allergy, the relative simplicity of Fel d 1 and the fact that 95% of cat-allergic subjects make IgE to this molecule have resulted in the selection of cat allergy as a model for the development and evaluation of this form of therapy. Four published studies evaluated the safety and efficacy of peptide immunotherapy for cat allergy using two polypeptides from Fel d 1. An equimolar mixture of the peptides (27 amino acids each in length) or placebo was administered in four subcutaneous injections (over a 2-week period) to 95 cat-sensitive patients in three dose groups (7.5, 75, and 750  $\mu\text{g}$  per injection) [26]. Statistically significant improvements in lung and nasal symptom scores were observed but only in the high-dose group. A prominent placebo effect was observed. Treatment was associated with a significant incidence of adverse events, which occurred a few minutes to several hours after peptide injection. Related *in vitro* studies demonstrated reduced IL-4 production in peptide-specific T-cell lines following therapy [27].

A reduction in IL-4 production was also reported by Pene and colleagues [28]. Peripheral blood mononuclear cells (PBMCs) were stimulated with cat dander extract *in vitro*, before and after treatment with the peptide mixture. A significant reduction in IL-4 production was observed in the high-dose group (subjects received individual doses of 750  $\mu\text{g}$  up to a total dose between 1500 and 4500  $\mu\text{g}$ ) but not in lower-dose groups. A reduction in allergen PD<sub>20</sub> (provocative dose of inhaled allergen resulting in a 20% reduction in forced expiratory volume in 1 second [FEV<sub>1</sub>]) was also observed in response to high and medium (individual doses of 75  $\mu\text{g}$  up to a total dose between 150 and 450  $\mu\text{g}$ ) dose regimens when compared with baseline but not when compared with placebo.

In a double-blind, parallel group study using the same two peptides, Simons and colleagues [29] administered four weekly subcutaneous injections of 250  $\mu\text{g}$  of the peptide mixture or placebo to 42 subjects with cat-allergic rhinitis or asthma. Treatment was associated with late onset symptoms of rhinitis, asthma, and pruritus. In contrast to other studies with the same peptide preparations, PBMC cytokine secretion profiles were no different in peptide-treated and placebo-treated subjects. No changes in early and late phase skin responses to whole allergen challenge were recorded.

In a multicenter study, 133 cat-allergic individuals received eight subcutaneous injections of 750  $\mu\text{g}$  of the same mixture of two Fel d 1 peptides. A significant improvement in pulmonary function was observed but only in individuals with reduced baseline FEV<sub>1</sub> [30]. Improvements in lung function were evident at a single time point (3 weeks) after therapy, and no other significant treatment responses were observed. Several allergic reactions were reported during treatment, including some requiring epinephrine. In common with other studies using these peptides, late onset adverse reactions diminished with successive doses of peptide, indicating that a degree of tolerance had been induced.

More recently, a series of clinical studies have been performed using mixtures of shorter peptides from Fel d 1 [31–36]. Cat-allergic asthmatic volunteers were challenged intradermally with whole cat dander allergen extract before and after a single injection of 5  $\mu\text{g}$  of each of 12 peptides in saline. The peptides spanned approximately 80% of the Fel d 1 molecule and encompassed most of the major T-cell epitopes. Peptide administration significantly reduced the magnitude of the cutaneous late phase reaction to intradermal allergen challenge. Evaluation of PBMC responses to allergen *in vitro* revealed a reduction in allergen-specific proliferation and a reduction in Th2 cytokines and IFN- $\gamma$  [31].

Fel d 1 peptide immunotherapy was subsequently evaluated in a small double-blind, placebo-controlled clinical trial [32]. Twenty-four cat-allergic asthmatic subjects with moderate-to-severe asthma were subjected to cutaneous allergen challenge, inhaled methacholine PC<sub>20</sub>, and inhaled allergen PD<sub>20</sub>. Quality of life was evaluated by questionnaire (global evaluation to cat exposure). The primary outcome measure of the study was the magnitude of the late phase cutaneous reaction to intradermal challenge with allergen extract. Secondary outcome measures included the early phase cutaneous reaction to allergen challenge, the allergen PD<sub>20</sub>, and the histamine PC<sub>20</sub>. Baseline measurements were compared with two posttreatment follow-up evaluations. The first of these was performed 4 to 8 weeks after therapy and the second 3 to 9 months after peptide therapy. Subjects received a total of 90  $\mu\text{g}$  of each of 12 peptides in divided incremental doses starting at 5  $\mu\text{g}$  and administered at 3- to 4-day intervals. Peptide treatment resulted in a statistically significant reduction in the magnitude of early (second follow-up only) and late phase cutaneous reactions (both follow-up assessments) to intradermal challenge with allergen when compared with placebo. Investigation of peripheral blood responses to allergen before and after therapy demonstrated reduced proliferative responses and reduced Th1 and Th2 cytokine

production. The reductions in proinflammatory cytokines were associated with an increase in production of IL-10. Subjects treated with peptides felt significantly better able to tolerate exposure to cats after therapy, although this improvement was not statistically significant when compared with responses from the placebo group. In this study of a small number of individuals, no significant improvements were observed in PD<sub>20</sub> or PC<sub>20</sub>.

In a small open-label study using a similar peptide preparation delivered at 2-week intervals rather than 3- to 4-day intervals, a significant reduction in airway hyperreactivity (measured by PC<sub>20</sub>) was observed [33]. Peptides were administered by intradermal injection as in previous studies; however, a lower dosing regimen was applied (0.1, 1.0, 5, 10, and 25 µg). In addition to an improvement in nonspecific airway hyperreactivity, a significant reduction in the magnitude of the cutaneous late phase reaction was observed following allergen challenge in the skin, in keeping with earlier studies. Immunohistochemistry of skin tissue biopsy obtained after allergen challenge revealed a significant increase in the number of CD25+ cells when compared with the number after placebo challenge after peptide immunotherapy. The number of CD4+/IFN-γ+ cells increased significantly after peptide treatment, suggesting that recruitment of Th1 cells to the skin may be an important mechanism. No increases in IL-10+ cells were observed in the skin, but expression of transforming growth factor-β (TGF-β) mRNA appeared to be elevated. For technical reasons, the identity of the cells producing TGF-β could not be determined.

A related study was performed to investigate the effect of peptide immunotherapy on peripheral blood CD4+ responses and CD4+ CD25+ suppression of allergen-stimulated cultures in a double-blind, placebo-controlled trial [35]. Proliferative responses and IL-13 production from PBMCs cultured with allergen *in vitro* were significantly reduced following peptide therapy as in previous studies. The functional regulatory activity of CD4+ CD25+ cells was assessed by mixing with autologous CD4+ CD25- cells. Peptide immunotherapy did not alter the suppressive activity of CD4+ CD25+ cells in this study, suggesting that naturally occurring regulatory T cells may not have a significant role in the immunologic changes associated with peptide immunotherapy.

The role of antigen-specific inducible regulatory T cells (rather than naturally occurring CD4+ CD25+ cells) was addressed in a subsequent study by mixing CD4+ T cells (the population of cells containing the putative regulatory cells) with CD4- cells [36]. Each population was labeled with a different color fluorescent dye. CD4+ cells were labeled red, whereas CD4- cells were labeled green with cell cycle tracking dye. In an autologous culture system, CD4+ cells from before and after peptide therapy were mixed with CD4- cells from before and after therapy, in all possible combinations. The results demonstrated that antigen-specific proliferative responses of memory T cells were reduced following peptide immunotherapy when compared with baseline samples, and that CD4+ cells isolated after treatment could suppress the proliferative response of baseline CD4- cells. These data suggest that peptide immunotherapy can induce a population of CD4+ T cells with suppressive-regulatory activity.

*Insect venom allergy*

Phospholipase A<sub>2</sub> (Api m 1) peptides were evaluated in a small open study in which five bee venom-allergic subjects received divided incremental doses of an equimolar mixture of three immunodominant peptides at weekly intervals [37]. Ten subjects treated with conventional bee venom immunotherapy served as a control group. The cumulative peptide dose was 397.1 µg. One week after the last peptide injection, subjects were challenged subcutaneously with 10 µg of whole Api m 1. All five subjects tolerated the challenge without systemic allergic symptoms. One week later, a wild bee sting challenge was performed. Three of five subjects tolerated this challenge without reaction, whereas the remaining two subjects sustained mild systemic allergic reactions. No change was observed in levels of allergen-specific serum IgE or IgG4 during the course of peptide therapy; however, following subcutaneous challenge with the whole allergen 1 week after the last peptide injection, concentrations of both isotypes increased sharply, particularly IgG4. Three to 4 weeks later, serum levels of specific IgG4 were higher than IgE.

Texier and colleagues [38] defined potentially immunodominant T-cell epitopes in Api m 1 by direct binding of peptides to purified MHC class II molecules. Four peptides were identified, three of which were similar to those used previously for therapy by Müller and colleagues. Following a similar clinical protocol, Tarzi and colleagues [39] performed a controlled, open-label, single-blind study of peptide therapy in subjects with mild bee venom allergy. Peptide therapy was well tolerated, and no allergic reactions were observed during treatment. Peripheral blood responses to allergen in vitro showed reduced proliferation of T cells to purified allergen and whole bee venom. Proliferative responses to treatment peptides were also reduced. Th2 cytokine production following culture with allergen was reduced but associated with a concomitant increase in IL-10. Late phase cutaneous reactions to whole bee venom and Api m 1 were significantly reduced following allergen challenge. Serum samples were collected before, during, and after peptide therapy. Allergen-specific IgG, IgG4, and IgE levels were measured at the conclusion of the study. The results revealed a significant but transient increase in allergen-specific IgG and IgG4 following peptide immunotherapy. The functional activity of such an increase remains to be determined.

Using a rush desensitization protocol, Fellrath and colleagues [40] treated bee venom-allergic subjects using three synthetic polypeptides (long synthetic peptides) covering the whole Api m 1 molecule. Patients received approximately 250 µg in incremental divided doses at 30-minute intervals starting with 0.1 µg. Maintenance injections of 100 µg, or in some cases 300 µg, were given on days 4, 7, 14, 42, and 70. A transient increase in T-cell proliferation to the peptides was observed during therapy in the active treatment group. IFN-γ and IL-10 levels but not Th2 cytokines increased. Allergen-specific IgG4 but not IgE levels increased throughout the study period. Peptide-specific IgE was induced in some patients during the study. No significant change in skin sensitivity to intradermal allergen

challenge was observed. Peptide therapy was generally well tolerated; however, local and disseminated erythema with occasional hand palm pruritus was observed in two subjects.

### **Mechanisms of peptide-induced tolerance**

Immunologic tolerance in the T-cell compartment is likely to arise through systemic presentation of peptides (which are delivered to the immune system in saline without adjuvant and at very low doses) to naïve T cells by nonprofessional antigen-presenting cells and steady state (quiescent) dendritic cells, both of which are known to induce tolerogenic T-cell responses [41]. Because the peptides are encountered in a noninflammatory environment, the T-cell response will be one of tolerance through expansion of existing allergen-specific regulatory T cells and *de novo* generation of such cells from the naïve T-cell pool. When peptides are administered by intradermal injection, a significant amount of the injected dose is likely to pass rapidly into the systemic circulation through the capillary bed. Once in the circulation, peptides rapidly reach all tissues and bind to MHC class II molecules of the appropriate specificity. A relatively low plasma dose and high solubility may render peptides “tolerogenic.” Recent studies in allergic disease have suggested that cross-linking of IgE on the surface of antigen-presenting cells (eg, by allergen) results in the activation of these cells and the release of cytokines [42]. Peptides may bind to MHC class II molecules on the cell surface without activating the cell. Previous studies have demonstrated the presence of a significant percentage of MHC class II molecules without bound peptides on the surface of immature and steady state dendritic cells [43]. Furthermore, this observation is associated with active HLA-DM, a chaperone and peptide-editing protein capable of loading exogenous peptides into empty MHC class II binding grooves [44]. As a result, when peptide-loaded dendritic cells recycle through lymphoid tissue, they present peptides to T cells while in a quiescent state, resulting in a tolerogenic encounter.

### **The role of peptide dose in therapy**

Little is known about the most effective dose for induction of tolerance through peptide therapy. In mice, tolerogenic peptide doses range from a few micrograms [45] to milligrams [46]. By delivering T-cell epitopes directly to dendritic cells *in vivo*, it has recently been shown that doses as small as 500 pg can induce tolerance in a murine model [47]. Fundamental differences may exist in the mechanisms of low- and high-dose tolerance. High-dose protocols have been associated with clonal deletion and, to a lesser extent, anergy of antigen-specific cells [6,46,48]. Fewer studies of low-dose tolerance have been per-

formed, but both high- and low-dose models appear to be characterized by the induction of T cells with regulatory activity [49–51].

The route of administration, physical properties, and solubility of the peptide are likely to influence whether tolerance or T-cell activation occurs. Microgram doses of allergen peptides administered intradermally resulted in systemic manifestations of tolerance such as reduced skin reactivity to allergen [31,32]. The same preparation delivered by inhalation (nebulized in saline and inhaled orally) did not induce tolerance [52]. This observation suggests that the route of administration can determine tolerogenicity. A threshold plasma dose of peptide may be required to establish systemic tolerance. Oral or inhaled delivery of peptide may require higher doses than the intradermal or intravenous routes to achieve efficacy. Indeed, a recent study in which tolerance was achieved through oral peptide administration supports this conclusion [23].

## Summary

Synthetic peptides representing T-cell epitopes of allergens and autoantigens have been employed to induce antigen-specific tolerance in vivo in experimental models and the clinical setting. Delivery of peptides orally or by injection leads to reduced reactivity to antigen accompanied by the induction of T cells with a regulatory phenotype. In most models, production of IL-10 (and occasionally TGF- $\beta$ ) has been increased, whereas PBMC secretion of Th1 and Th2 cytokines is reduced. Peptide therapy may provide a safe, effective, and economically viable approach for disease-modifying therapy in autoimmune and allergic diseases.

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