

Review article

Peptide immunotherapy for allergic diseases

Specific allergen immunotherapy has been widely practised for almost 100 years. Whilst this approach is disease-modifying and efficacious, the use of whole allergen preparations is associated with an unacceptably high prevalence of allergic adverse events during treatment. Many approaches to reduce the allergenicity of immunotherapy preparations whilst maintaining immunogenicity are under development. One such approach is the use of short synthetic peptides which represent major T-cell epitopes of the allergen. Major potential advantages of this approach include markedly reduced capacity to cross-link immunoglobulin-E and activate mast cells and basophils, and ease of manufacture and standardization. Promising results in preclinical studies have led to the translation of this approach to clinical studies in humans. Peptide immunotherapy is currently under development for allergic and autoimmune diseases.

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Introduction

Recent evidence suggests that immune responses to allergens consist of both inflammatory and regulatory components (1). The balance between these elements of immunity determines whether clinical tolerance or allergy result. The reasons why some individuals suffer from allergic diseases, whilst other equivalently exposed individuals do not, are far from clear. Both genetic and environmental factors influence susceptibility. Analysis of genes associated with allergic (2) diseases suggests that susceptibility arises from a complex interaction between multiple genes, with many elements controlling the immune response being polymorphic.

The marked recent increase in the prevalence of allergic sensitization during recent decades provides evidence for the additional role of environmental factors in the pathogenesis of allergic disease. It was initially proposed that the increasing prevalence of allergy in wealthier societies may be associated with improved sanitation, vaccination, use of antibiotics and exposure to fewer infectious organisms (3). However, this Hygiene Hypothesis could not account for parallel increases in the prevalence of Th1-mediated autoimmune diseases such as type I diabetes (4). The original 'hygiene hypothesis' focused on a balance and mutual antagonism between Th1 (pro-inflammatory) and Th2 (pro-allergic) immunity,

and is likely to have been an oversimplification. Studies in experimental models of allergic airway disease have shown that induction of a Th1 response, in the presence of Th2 pathology, leads to increased disease severity (5). Furthermore, Th2 cells have also been associated with autoimmune diseases (6).

In recent years it has become clear that numerous populations of cells with immunoregulatory properties exist. These cells appear to be crucial in homeostatic regulation of inflammatory responses. Several populations of regulatory T cells have been characterized including 'natural' CD25⁺, FoxP3⁺ cells and additional subsets of Th3 cells producing transforming growth factor- β (TGF- β) and Tr1 regulatory cells producing interleukin-10 (IL-10) (7). 'Natural' regulatory T cells arise either in the thymus or peripheral lymphoid organs, whereas Th3 and Tr1 cells appear to arise from naïve lymphocytes in the periphery. Deficits in the functional activity of CD4⁺CD25⁺ and Tr1 subsets of regulatory cells have been reported in both allergic (8–10) and autoimmune diseases (11–13).

Many therapeutic approaches for allergic disorders are palliative rather than disease modifying and tend to cause nonspecific immunosuppression. Specific immunotherapy (SIT) is a form of disease-modifying treatment that has been demonstrated to be clinically efficacious in both allergic rhinitis and asthma (14–16). SIT reduces

subsequent allergic sensitization (17) and appears to reduce the incidence of asthma in later life (18). Subcutaneous administration of increasing doses of the sensitizing allergen protein(s) modifies the immune response in a variety of ways. Mechanistic studies have shown downregulation of Th2 responses in peripheral blood (19–21), and/or increased Th1 responses in the tissue (20, 22). SIT is also associated with the induction of strong allergen-specific immunoglobulin-G (IgG) responses to allergen (23). Increased Th1 : Th2 ratio accompanied by induction of IgG initially led to the conclusion that the efficacy of SIT was achieved through the induction of 'protective' Th1 responses which antagonized Th2 immunity. More recently an important role for IL-10 (and in some cases TGF- β) has been identified. Bee keepers exposed to multiple stings may develop local reactions to the sting at the beginning of the bee keeping season but these soon disappear. These individuals rarely suffer from severe allergic reactions. Protection appears to be afforded by strong allergen-specific T cell IL-10 responses and specific IgG₄. The fact that IL-10 acts as an isotype switch factor for IgG₄ provides a mechanistic link between T-cell and B-cell response (24). Such findings together with current interest in regulatory populations of T cells, has recently resulted in a re-examination of mechanisms in SIT. Several studies have now documented the presence of increased numbers of cells positive for IL-10 (and in some cases TGF- β) mRNA or protein in the peripheral blood (25–27) and tissues (28, 29) of treated individuals. Thus, a current focus of research is to identify strategies to safely and effectively induce regulatory cells in an antigen-specific fashion for the treatment of allergic disease.

Despite our improved understanding of the mechanisms of SIT, the fact remains that treating allergic

individuals with a sensitizing allergen molecule occasionally results in allergic adverse events. Rarely these can be fatal. Considerable resources have been invested in developing strategies to reduce the allergenicity of immunotherapy preparations whilst maintaining their ability to modify T-cell and/or B-cell responses. One such approach is to treat patients with synthetic peptides that correspond to the major T-cell epitopes of the allergen. An advantage of this approach is the avoidance of IgE-mediated mast cell/basophil activation and the presentation of allergen-derived T-cell epitopes by quiescent antigen-presenting cells (Fig. 1).

Preclinical experimental models

Immunological tolerance induced by either prophylactic or therapeutic treatment with peptides has been demonstrated in numerous murine models of autoimmunity and allergy. Early studies of peptide therapy (PIT) were performed in models of autoimmune disease. In experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis, high-dose intravenous peptide administration resulted in clonal deletion of T cells and protection against disease (30). EAE was also prevented by intraperitoneal administration of peptides from myelin basic protein (MBP) (31). In another model, intranasal, but not oral, administration of an MBP peptide resulted in protection from EAE (32). Most recently MBP peptides were given intranasally to T-cell receptor transgenic mice in an EAE model. Mice were protected from disease in a process that was dependent on IL-10 (33).

Several studies have demonstrated the efficacy of PIT in experimental arthritis. Peptides from type II collagen (34, 35) protected animals from collagen-induced arthritis

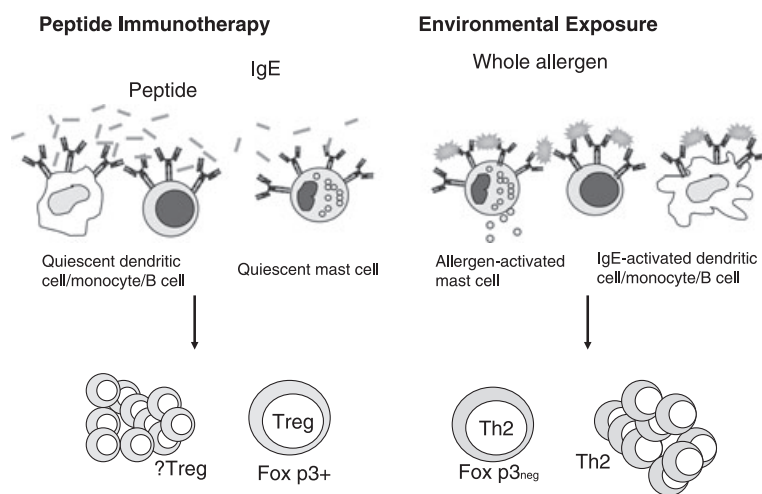


Figure 1. Mechanisms underlying T-cell responses to allergen following environmental exposure and peptide immunotherapy. In an allergic individual, antigen-presenting cells (APC) including dendritic cells, monocytes and allergen-specific B cells can all express allergen-specific IgE on their surfaces. Short peptides, unlike whole allergen, do not crosslink surface IgE (even though they may be capable of monomeric binding) and do not activate APC. Quiescent APC generate a regulatory T-cell response. Allergen-IgE-activated APC provide signals leading to the generation of pro-inflammatory Th2 cells and perpetuation of allergic inflammation.

whilst peptides from the heat shock protein (hsp) 60 protected mice from adjuvant arthritis (36).

Similarly, peptides from disease-related antigens such as the insulin B chain (37) and glutamate decarboxylase (GAD65) (38), or peptides from heat shock proteins (39), have been shown to prevent the development of type I diabetes in multiple murine models.

Evaluation of PIT in allergic disease models has been slower to develop. A variety of allergen sensitization models have been employed including perennial allergens such as Der p 2 and Fel d 1, seasonal allergens such as Bet v 1 and insect venom allergens such as Api m 1, Api m 4 and Dol m 5. Treatment of mice sensitized to Fel d 1 with two allergen-derived polypeptides resulted in decreased production of IL-2 and allergen-specific IgG (40). Intranasal delivery of Der p 2 peptides to house dust mite sensitized mice downregulated both T cell and antibody responses to the native protein (41). In a model of tree pollen allergy, the dominant T-cell epitope of the major birch pollen allergen Bet v 1 was administered to sensitized mice both before and after induction of allergic inflammation. T-cell proliferative responses were diminished by both treatments but no change in antibody response was observed (42). Prophylactic administration of peptides from the bee venom allergen Api m4, or the hornet venom allergen Dol m 5 to mice sensitized to whole venom allergens resulted in a partial reduction in both T-cell proliferation and B-cell antibody responses to subsequent allergen sensitization (43). In unrelated studies of venom allergy, mice were sensitized to Api m 1 and treated with a mixture of three polypeptides spanning the entire molecule. Mice were protected from anaphylaxis. A significant reduction in specific IgE and an increase in allergen-specific IgG2a was observed (44).

Clinical studies

Published clinical studies of peptide immunotherapy in allergic disease are restricted to peptides from two model allergens, Fel d1 from cats and Api m1 (PLA₂) from bee venom. The reported clinical and mechanistic outcomes of peptide immunotherapy are shown in Fig. 2. Studies are summarized in Table 1.

Cat allergy

A number of PIT studies have been performed in subjects allergic to cats. In four studies the safety and efficacy of a mixture of two polypeptides from Fel d 1 was evaluated. In the first of these to be reported, peptides were administered subcutaneously to 95 cat allergic subjects at 3- to 4-day intervals. Three peptide doses were employed (7.5, 75 and 750 µg per injection) (45). Improvements in lung and nasal symptom scores were observed at higher doses. Treatment was associated with a large number of adverse events occurring minutes to hours after peptide administration. Related *in vitro* studies showed reduced IL-4 production in peptide-specific T-cell lines *in vitro* following therapy (46).

In further studies, reduction in allergen PD₂₀ (provocative dose of inhaled allergen resulting in a 20% reduction in forced expiratory volume in 1 s; FEV₁) was observed in response to high-dose (individual doses of 750 µg up to a total dose of between 1500 and 4500 µg) and medium-dose (individual doses of 75 µg up to a total dose of between 150 and 450 µg) regimens when compared with baseline, but not when compared with placebo (47). Treatment was also associated with reduced allergen-induced IL-4 production from peripheral blood mononuclear cells (PBMC).

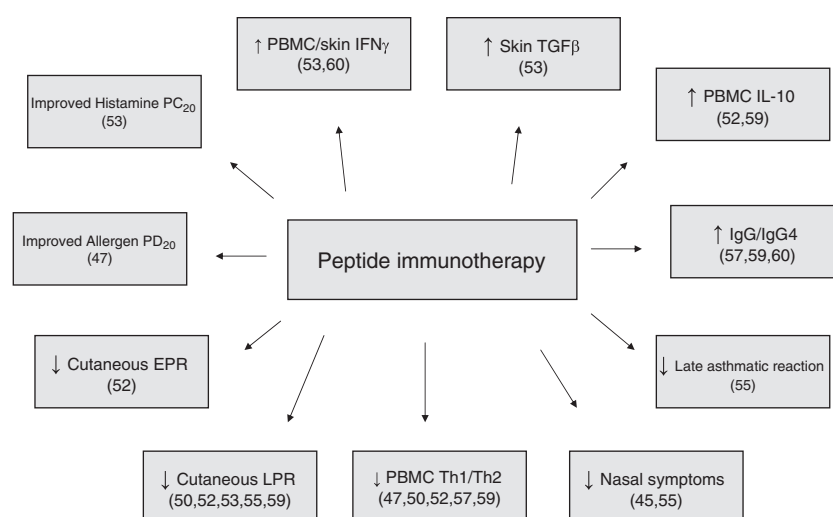


Figure 2. Clinical and mechanistic findings in peptide immunotherapy. Peptide immunotherapy has been performed using different peptide preparations, allergens and clinical protocols. The figure shows modified outcomes and references. The figure does not cite negative findings/studies.

Table 1. Clinical studies of peptide immunotherapy

Ref.	Allergen	Peptide number & length (residues)	Dose (μ g)	Route	Major outcome measures
Norman et al. (45)	Cat; Fel d1	2 $\times$ 27	30–3000	s.c.	Improved lung and nasal symptoms
Simons et al. (48)	Cat; Fel d1	2 $\times$ 27	1000	s.c.	No change in early & late-phase skin reactions following allergen challenge
Pene et al. (47)	Cat; Fel d1	2 $\times$ 27	15–4500	s.c.	Improved allergen PD ₂₀
Muller et al. (57)	Bee; Api m1 (PLA ₂)	3 : 11, 12, 18	397.1	s.c.	Protection from allergen challenge and partial protection from live bee sting
Maguire et al. (49)	Cat; Fel d1	2 $\times$ 27	6000	s.c.	Improved FEV ₁ in some subjects
Oldfield et al. (50)	Cat; Fel d1	12 $\times$ 16/17	5	i.d.	Reduced late-phase skin reaction following allergen challenge
Oldfield et al. (52)	Cat; Fel d1	12 $\times$ 16/17	90	i.d.	Reduced early & late-phase skin reaction following allergen challenge, Th1 + Th2 responses in PBMC reduced, IL-10 increased
Fellrath et al. (60)	Bee; Api m1 (PLA ₂)	3 : 45, 53, 60	751.1–1551.1	s.c.	Increased allergen-specific IgG4, increased IFN- γ and IL-10 in PBMC
Alexander et al. (53)	Cat; Fel d1	11 $\times$ 16/17	40.1	i.d.	Decreased AHR and skin late-phase reaction to allergen challenge
Alexander et al. (55)	Cat; Fel d1	12 $\times$ 16/17	131–341	i.d.	Improved nasal symptom scores, reduced late asthmatic reaction following allergen challenge
Tarzi et al. (59)	Bee; Api m1 (PLA ₂)	4 $\times$ 18	431.1	i.d.	Reduced late-phase skin reaction following allergen challenge, Th1 + Th2 responses in PBMC reduced, IL-10 increased transiently increased allergen-specific IgG4

In a double-blind, placebo-controlled, parallel-group study, peptides or placebo was administered weekly by subcutaneous injection (four doses of 250 μ g) to 42 subjects with cat-allergic rhinitis and/or asthma (48). Treatment was associated with adverse events, primarily late-onset symptoms of rhinitis, asthma and pruritis. PBMC cytokine secretion profiles did not differ between peptide-treated and placebo-treated subjects. No changes in early and late-phase skin responses to allergen challenge were observed.

In the largest of the four studies, 133 cat allergic subjects received eight subcutaneous injections of 750 μ g of the peptide mixture. The only significant clinical outcome was an improvement in pulmonary function observed in individuals with reduced baseline FEV₁ (49). This improvement was only evident at a single time point (3 weeks) after therapy. Several adverse events were reported in common with other studies.

More recently, a series of clinical studies have been performed using mixtures of shorter peptides from Fel d 1 (50–56). Peptides were administered intradermally to cat allergic asthmatic subjects of mild to moderate disease severity. Peptide administration significantly reduced the magnitude of the cutaneous late-phase reaction to intradermal allergen challenge. Evaluation of PBMC responses to allergen *in vitro* demonstrated reductions in both Th2 cytokines and γ -interferon (IFN- γ) (50).

Subsequently, in a double-blind, placebo-controlled study, 24 cat allergic asthmatic subjects underwent cutaneous allergen challenge, inhaled methacholine PC₂₀ and inhaled allergen PD₂₀ (52). Subjects received a total of 90 μ g of each of 12 peptides, covering most of the Fel d 1 molecule, in divided incremental doses starting at 5 μ g and administered at 3- to 4-day intervals. There was a significant reduction in both early and late-phase cutaneous reactions to intradermal challenge with allergen when compared with placebo. Reduced proliferative responses and Th1 and Th2 cytokine production were

observed in the active treatment group. Reductions in pro-inflammatory cytokines were associated with a concomitant increase in IL-10. Subjects treated with peptides felt significantly better able to tolerate exposure to cats after therapy. No significant improvements were observed in PD₂₀ or PC₂₀, but the study may not have been sufficiently powered to detect such differences which were secondary, rather than primary, outcome measures.

However, a significant improvement in PC₂₀ was observed in a small open-label study using a similar peptide preparation delivered at 2-week intervals rather than 3- to 4-day intervals (53). Peptides were administered by intradermal injection but at 2-week intervals and a lower dosing regimen was applied (total dose: 41.1 μ g). A significant reduction in the magnitude of the cutaneous late-phase reaction was also observed following allergen challenge in the skin. There was a significant increase in the number of CD25⁺ cells in allergen challenge skin sites compared with placebo challenge after peptide immunotherapy. The number of CD4⁺/IFN- γ ⁺ cells also increased, 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 TGF- β mRNA was increased.

Studies evaluating upper and lower airway outcomes following a higher dose PIT protocol have also been performed (55). Cat allergic asthmatic subjects were screened by inhaled incremental allergen challenge to establish whether they developed a single early asthmatic response or a dual early and late asthmatic response. Those with dual responses were recruited into a small double-blind, placebo-controlled ($n = 16$; 8 active, 8 placebo) study whilst those displaying single early responses ($n = 12$) were recruited into an open study. Both groups underwent the same treatment protocol receiving approximately 300 μ g of each of 12 peptides delivered as a mixture intradermally in saline. Outcomes were evaluated 4–8 weeks after the completion of therapy

and again at 3–4 months. Treatment significantly reduced cutaneous late-phase reactions to intradermal allergen challenge as in previous studies. The effect of PIT on late asthmatic responses was evaluated by comparison of bolus inhaled allergen challenge before and after treatment. No effect was observed on the early asthmatic reaction but a significantly reduced late asthmatic reaction was observed within the treatment group (FEV₁; area under curve 2–8 h postchallenge). In the open study allergen PD₂₀ was evaluated by incremental inhaled allergen challenge. Nasal outcomes were measured after inhaled allergen challenge. No change was observed in allergen PD₂₀ nor histamine PC₂₀. However, nasal allergen challenge revealed a significant reduction in outcome scores (sneezing, weight of nasal secretions and nasal blockage). Additionally, significant improvements were observed in three of seven rhinitis quality of life questionnaire fields.

The effect of peptide immunotherapy on peripheral blood CD4⁺ responses and CD4⁺CD25⁺ regulatory T-cell suppression of allergen-stimulated cultures has also been evaluated (54). Proliferative responses and IL-13 production from PBMC cultured with allergen *in vitro* were significantly reduced following PIT as in previous studies. Regulatory T-cell activity was assessed by mixing with autologous CD4⁺CD25⁺ cells. No change in the suppressive activity of CD4⁺CD25⁺ cells was observed. Thus, CD4⁺CD25⁺ regulatory T cells may not play a significant role in the mechanism of action of peptide immunotherapy.

The role of PIT on antigen-specific inducible regulatory T cells was addressed by mixing CD4⁺ T cells with CD4^{negative} cells (56). The two populations were labelled with different fluorescent dyes (CD4⁺ were labelled red and CD4^{negative} were labelled green). CD4⁺ cells from before and after PIT were mixed with CD4^{negative} cells from before and after therapy, in all possible combinations. The results demonstrated that CD4⁺ cells isolated after PIT could suppress the proliferative response of baseline CD4^{negative} cells. These data provide evidence that peptide immunotherapy induces a population of CD4⁺ T cells with regulatory activity.

Insect venom allergy

The first study of PIT in insect venom allergic individuals employed peptides from Api m 1. Five bee venom allergic subjects received divided incremental doses of a mixture of three peptides at weekly intervals (57). Ten subjects treated with conventional bee venom immunotherapy served as controls. A cumulative peptide dose of 397.1 µg was given. Subcutaneous challenge with 10 µg of whole Api m 1 was tolerated without systemic allergic symptoms by all subjects. Three of five tolerated a wild bee sting challenge without reaction, the remaining two subjects developed mild systemic allergic reactions. No changes were observed in levels of allergen-specific serum IgE or

IgG₄ during PIT but following subcutaneous challenge with whole allergen 1 week after the last peptide injection, serum concentrations of both antibody isotypes increased sharply.

Texier et al. determined the major T-cell epitopes of Api m 1 using direct binding of peptides to purified major histocompatibility complex class II molecules (58). Four peptides were identified, which were administered to bee venom allergic individuals with mild disease in a controlled, open-label, single-blind study (59). A total dose of 431.1 µg of each of the four peptides was administered to 12 individuals. Treatment was well tolerated and no adverse events were observed. Allergen-specific T-cell proliferative responses to whole bee venom and purified allergen were reduced following treatment. IL-13 and IFN-γ responses of PBMC cultured with allergen were reduced after treatment whereas IL-10 was increased. Late-phase cutaneous reactions to both whole bee venom and Api m 1 were significantly reduced. There was a small, significant but transient increase in allergen-specific IgG and IgG₄ during peptide immunotherapy. No subjective outcome measures were analysed.

In another study, bee venom allergic subjects were treated according to a RUSH protocol with three synthetic polypeptides (long synthetic peptides) covering the whole Api m 1 molecule and ranging in length from 45 to 60 amino acids in length (60). Patients received approximately 250 µg in incremental divided doses at 30 min intervals starting with 0.1 µg. Up to five maintenance injections of 100 µg, or in some cases 300 µg, were subsequently given. No significant change in skin sensitivity to intradermal allergen challenge was observed. 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 levels increased throughout the study period. Peptide-specific IgE was induced in some patients during the study. Some local and disseminated erythema with occasional hand palm pruritis was observed in two subjects.

In conclusion, improved understanding of the mechanisms of immunological tolerance and the mechanisms underlying the efficacy of SIT have allowed substantial progress to be made in peptide immunotherapy. There are numerous studies in both allergic and autoimmune diseases that support the potential of this approach. More studies are required to define the optimal dose range, dose interval and route of administration. Initial concerns over the incidence of adverse events may be allayed through use of short peptides and low doses.

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