ORIGINAL ARTICLE

Immunotherapy with Allergen Peptides

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Specific allergen immunotherapy (SIT) is disease-modifying and efficacious. However, the use of whole allergen preparations is associated with frequent allergic adverse events during treatment. Many novel approaches are being designed to reduce the allergenicity of immunotherapy preparations whilst maintaining immunogenicity. One approach is the use of short synthetic peptides which representing dominant T cell epitopes of the allergen. Short peptides exhibit markedly reduced capacity to cross link IgE and activate mast cells and basophils, due to lack of tertiary structure. Murine pre-clinical studies have established the feasibility of this approach and clinical studies are currently in progress in both allergic and autoimmune diseases.

Key words: *allergy, epitope, IL-10, immunological tolerance, immunotherapy, peptide, regulatory T cell, T cell*

Specific allergen immunotherapy (SIT) is disease modifying and efficacious. However, the use of whole-allergen preparations is associated with frequent allergic adverse events during treatment. Many novel approaches are being designed to reduce the allergenicity of immunotherapy preparations while maintaining immunogenicity. One approach is the use of short synthetic peptides that represent dominant T-cell epitopes of the allergen. Short peptides exhibit markedly reduced capacity to cross-link immunoglobulin (Ig)E and activate mast cells and basophils owing to a lack of tertiary structure. Murine preclinical studies have established the feasibility of this approach, and clinical studies are currently in progress in both allergic and autoimmune diseases.

In non-allergic individuals, allergen exposure can be associated with a failure to mount a detectable immune response. In those individuals who do make an immune response, it is characterized by non-inflammatory ''regulatory'' elements, such as interleukin (IL)-10-secreting T cells.[1] The reasons why some individuals suffer from allergic diseases and others do not despite equivalent exposure are far from clear. Genetic and environmental factors influence susceptibility. Analysis of genes associated with allergic diseases suggests that susceptibility arises from a complex interaction between multiple (frequently polymorphic) genes.[2]

A role for environmental factors in the pathogenesis of allergic disease is demonstrated by the recent rise in the prevalence of allergic sensitization and disease in industrialized countries. Changes in sanitation, diet, vaccination practices, and other facets of modern life have been linked to increases in the prevalence of both allergic and autoimmune disease, which probably arise as a result of deficient immune regulation.[3]

Several populations of cells with immunoregulatory properties exist. They are important in homeostatic regulation of inflammatory responses. A number of populations of regulatory T cells have been characterized, including 'natural' $CD4^+CD25^+FoxP3^+$ cells and additional subsets of T helper (Th)3 cells producing transforming growth factor (TGF) β and Tr1 regulatory cells producing IL-10.[4] ''Natural'' regulatory T cells arise either in the thymus or peripheral lymphoid organs, whereas Th3 and Tr1 cells appear to arise from naive lymphocytes in the periphery. Deficits in the functional activity of $CD4^+CD25^+$ and Tr1 subsets of regulatory cells have been reported in both allergic[5–8] and autoimmune[9–11] diseases.

SIT, through administration of allergen, is a form of disease-modifying treatment that has been demonstrated to be clinically efficacious in allergic rhinitis and asthma and to provide enduring clinical benefit.[12–14] SIT reduces

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subsequent allergic sensitization to other allergens[15] and reduces the incidence of asthma in later life.[16,17] Mechanistic studies have shown down-regulation of Th2 responses in peripheral blood[18–20] and/or increased Th1 responses in the tissue.[19,21] SIT is also associated with the induction of allergen-specific IgG to allergen.[22] An increased Th1 to Th2 ratio accompanied by induction of IgG initially suggested that efficacy was achieved through the induction of a "protective" Th1 responses, which antagonized Th2 allergen-specific responses. 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 allergic reactions at the beginning of the bee-keeping season, but these gradually disappear over a period of days. Protection is associated with strong allergen-specific T-cell IL-10 responses and specific IgG4. IL-10 in combination with IL-4 drives IgG4 and provides a mechanistic link between T-cell and B-cell responses.[23] Several studies have recently documented increased numbers of cells expressing IL-10 (and in some cases TGF-β) messenger ribonucleic acid (mRNA) or protein in the peripheral blood[24–26] and tissues[27,28] of treated individuals. Strategies are being developed to induce regulatory cells in an antigen-specific fashion for the treatment of allergic disease and perhaps, in the future, autoimmune diseases.

Despite the efficacy of SIT, administration of whole-allergen molecules carries the risk of adverse events, which may be local or systemic and include life-threatening anaphylaxis. Considerable resources have been invested in developing strategies to reduce the allergenicity of immunotherapy preparations while maintaining their ability to modify T-cell and/or B-cell responses. One approach is to treat patients with synthetic peptides representing the immunodominant T-cell epitopes of the allergen. Short peptides have the advantage of being unable to cross-link allergen-specific IgE, leading to mast cell and basophil activation (Figure 1).

Preclinical Experimental Models

Numerous murine models have been developed of both allergic and autoimmune diseases that demonstrate the efficacy of peptide immunotherapy. Prophylactic and therapeutic protocols are effective. High-dose intravenous administration of peptide in experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis, resulted in clonal deletion of T cells and protection against disease.[29] Intraperitoneal administration of peptides from myelin basic protein (MBP) has also been shown to prevent EAE.[30] MBP peptides administered intranasally to T-cell receptor transgenic mice protected them from EAE

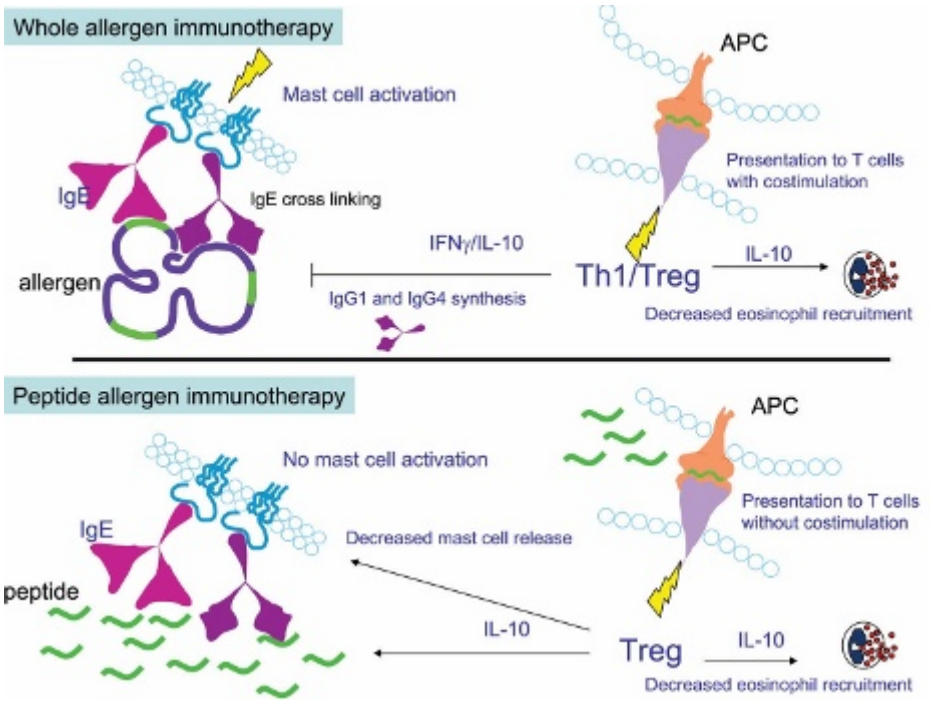

Figure 1. Comparison of whole-allergen immunotherapy and peptide immunotherapy. Whole-allergen immunotherapy leads to the generation of both T helper 1 (Th1) and T regulatory (Treg) responses. Interleukin-10 (IL-10) and interferon-γ (IFN-γ) produced by T cells of treated individuals reduce eosinophil recruitment. IL-10, IFN-γ, and IL-4 drive production of allergen-specific immunoglobulin (Ig)G antibodies. Peptides are presented to T cells with costimulation leading to a mixed Th1-Treg response. Whole-allergen molecules can cross-link allergen-specific IgE on the surface of mast cells and basophils, leading to cellular activation and IgE-mediated adverse events. APC = antigen-presenting cell. In peptide immunotherapy, short peptides do not cross-link allergen-specific IgE molecules and thus mast cells and basophils are not activated. Peptides are recognized by T cells in the absence of costimulation, resulting in a predominantly regulatory response characterized by IL-10, which decreases eosinophil recruitment and mast cell activation.

in a process that was IL-10 dependent.[31] Similarly, in arthritis models, peptides from type II collagen[32,33] protected animals from collagen-induced arthritis, whereas peptides from the heat shock protein 60 protected mice from adjuvant arthritis.[34] In diabetes models, peptides from the insulin B chain,[35] glutamate decarboxylase (GAD65),[36] and heat shock proteins[37] have been shown to prevent the development of disease.

Peptide immunotherapy has also been evaluated in models of allergic disease. Treatment of mice sensitized to Fel d 1 with two long peptides resulted in decreased production of IL-2 and allergen-specific IgG.[38] Curiously, no allergy-related end points were evaluated. Intranasal delivery of Der p 2 peptides to sensitized mice down-regulated both T-cell and antibody responses to the native protein.[39] In a model of birch pollen allergy, a dominant T-cell epitope of Bet v 1 was used to treat sensitized mice prophylactically and therapeutically following induction of allergic inflammation.[40] Administration of peptides from the bee venom allergen Api m4 or the hornet venom allergen Dol m 5 to mice prior to sensitization with whole-venom allergens caused a partial reduction in T-cell proliferation and antibody production.[41] In separate studies, mice were protected from anaphylaxis by treatment with a mixture of three long peptides from Api m1.[42]

Clinical Studies

Cat Allergen: Fel d 1

Several clinical studies of therapy with peptides from Fel d 1 have been reported in the last decade (Table 1). In four related studies, the safety and efficacy of a mixture of two long (27 amino acids each) peptides were evaluated. In the first of these, peptides were administered subcutaneously to 95 cat-allergic subjects at weekly intervals at three doses (7.5, 75, and 750 μg per injection).[43] At higher doses, improvements in lung and nasal symptom scores were observed. However, treatment was associated with frequent adverse events occurring minutes to hours after

Table 1. Clinical studies of peptide immunotherapy in allergy.

Allergen	*Peptide Characteristics*	*Study Design*	*Number of Subjects*	*Route of Administration*	*Total Dose (μg)*	*Clinical Outcomes*	*Reference*
Fel d 1 (cat)	2 × 27mer	DBPC	95	SC	30–3,000	Nasal and lung symptoms	43
	2 × 27mer	Open		SC	150–4,500	Allergen PD_{20}	46
	2 × 27mer	DBPC	42	SC	1,000	End-point titration, skin LPR	47
	2 × 27mer	DBPC	133	SC	600–6,000	FEV_1,* daily peak flow, skin EPR, symptom assessment	48
	3 × 16/17mer	Open	6	ID	80	Isolated LAR	44
	12 × 16/17mer	Open	8	ID	5	Isolated LAR and skin LPR	49
	12 × 16/17mer	DBPC	24	ID	90	Skin LPR and EPR, PC_{20}, PD_{20}	50
	11 × 16/17mer	Open	8	ID	41.1	Skin LPR, PC_{20}	51
	12 × 16/17mer	DBPC/open	28	ID	216–341	Nasal allergen challenge, bronchial challenge, skin LPR	53
Api m 1 (PLA_2) (bee)	1 × 11, 1 × 12, 1 × 18	Open	5	SC	397.1	Skin challenge PLA_2, bee sting	55
	1 × 60, 1 × 53, 1 × 45	DBPC	16	SC	751.1	End-point skin titration	58
	4 × 18	Open-controlled	24	ID	431.1	Skin LPR	57

DBPC = double-blind placebo controlled; EPR = early-phase reaction; FEV_1 = forced expiratory volume in 1 second; ID = intradermal; LAR = late asthmatic reaction; LPR = late-phase reaction; PC_{20} = provocative concentration of histamine that induces a 20% reduction in FEV_1; PD_{20} = provocative dose of inhaled allergen resulting in a 20% reduction in forced expiratory volume in 1 second; PLA_2 = phospholipase A_2; SC = subcutaneous.
*Only in subjects with reduced baseline FEV_1 and only at one time point.

peptide administration. Immediate reactions appeared to be IgE mediated and may have arisen as a result of cross-linking pre-existing peptide-specific IgE present in some patients or through the development of de novo peptide-specific IgE, as observed in others. The relatively large peptides employed (27 amino acids) may have retained conformational IgE epitopes or formed dimmers through disulphide binding. Later adverse events included what may have been isolated late asthmatic reactions, which were later characterized in detail.[44] Related in vitro studies showed reduced IL-4 production in peptide-specific T-cell lines in vitro following therapy.[45]

A further study evaluated allergen sensitivity by inhaled challenge of allergic asthmatic subjects before and after peptide therapy. Again, three dosage groups were used. Treatment was also associated with reduced allergen PD_{20} (provocative dose of inhaled allergen resulting in a 20% reduction in forced expiratory volume in 1 second [FEV_1]) in the high-dose and medium-dose groups, together with reduced allergen-induced IL-4 production from peripheral blood mononuclear cells (PBMCs).[46]

Not all studies demonstrate a positive clinical response. Peptides or placebo was administered weekly by subcutaneous injection (four doses of 250 μg) to 42 subjects with cat-allergic rhinitis and/or asthma.[47] Treatment was associated with frequent adverse events, mostly respiratory in nature. PBMC cytokine secretion patterns were not different in peptide-treated and placebo-treated subjects. No changes in allergen-induced early- and late-phase skin responses were observed.

In the largest of the four studies, 133 cat-allergic subjects received eight subcutaneous injections of 750 μg of the peptide mixture. The only significant clinical outcome was observed in a secondary analysis as an improvement in pulmonary function observed in individuals with reduced baseline FEV_1; the improvement was evident only at a single time point (3 weeks). Adverse events were common as in the other related studies.[48]

More recently, studies have been performed using mixtures of shorter peptides from Fel d 1.[44,49–54] Peptides were administered intradermally to cat-allergic asthmatic subjects of mild to moderate disease severity. Treatment with a single dose (5 μg of each peptide in a mixture) resulted in significant reductions in the magnitude of the cutaneous late-phase reaction to intradermal allergen challenge. PBMC cultures stimulated with allergen in vitro demonstrated reductions in both Th1 and Th2 cytokines.[49]

Subsequently, in a double-blind, placebo-controlled study, 24 cat-allergic asthmatic subjects were treated with incremental, divided doses of peptides (total dose 90 μg of each peptide).[50] Treatment resulted in a significant reduction in both early- and late-phase cutaneous reactions to allergen challenge when compared with placebo. Proliferative responses and Th1 and Th2 cytokine production from PBMCs cultured with allergen were also reduced in the active treatment group. Additionally, levels of IL-10 production were increased. Peptide treatment resulted in a significantly improved ability to tolerate exposure to cats after therapy. No significant improvements were observed in PD_{20} or PC_{20}.

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 improvement in PC_{20} was observed.[51] Peptides were given by intradermal injection with a 2-week interval and a lower total dose of peptide was administered (41.1 μg of each peptide). The cutaneous late-phase reaction was significantly reduced following allergen challenge in the skin. Significantly more $CD25^+$ cells were found in allergen challenge skin sites from peptide-treated subjects compared with placebo. The number of $CD4^+$/IFN-γ^+ cells also increased, suggesting that recruitment of Th1 cells to the skin may play a role in modifying the Th1:Th2 balance in the response to allergen. No increases in IL-10^+ cells were observed in the skin, but expression of TGF-β mRNA was increased.

Modulation of $CD4^+CD25^+$ regulatory T-cell function has been evaluated in allergen-stimulated cultures in a double-blind, placebo-controlled trial of peptide immunotherapy.[52] Proliferative responses and IL-13 production from PBMCs cultured with allergen in vitro were significantly reduced following peptide therapy as in previous studies. However, no improvement 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 effect of peptide therapy on non-$CD4^+CD25^+$ regulatory T cells was also investigated. The induction of allergen-specific ''inducible'' regulatory T cells was addressed by mixing $CD4^+$ T cells with $CD4^-$ cells before and after therapy.[54] The results demonstrated that $CD4^+$ cells isolated after therapy could suppress the proliferative response of baseline $CD4^-$ cells. These data provide evidence that peptide immunotherapy induces a population of $CD4^+$ T cells with allergen-specific regulatory or suppressive activity.

Insect Venom Allergy: Api m1

Fewer clinical studies have been reported with peptides from the major bee venom allergen Api m1 (phospholipase

A_2). In a small open study, five bee venom–allergic subjects received subcutaneous incremental doses of a mixture of three immunodominant peptides at weekly intervals.[55] The cumulative peptide dose was 397.1 μg, with a first dose of 0.1 μg building to a final series of maintenance doses of 100 μg. One week after completion of peptide dosing, subjects were challenged by subcutaneous administration of 10 μg of whole Api m 1. All subjects tolerated Api m 1 challenge without systemic allergic symptoms. One week later, a bee sting challenge was performed. Three individuals tolerated the challenge without any allergic sequelae; the remaining two subjects developed mild systemic allergic reactions. Levels of allergen-specific serum IgE or IgG4 did not change during the course of peptide therapy. However, following subcutaneous challenge with Api m 1 1 week after the last peptide injection, concentrations of both isotypes, in particular IgG4, increased markedly.

Immunodominant T-cell epitopes have been defined by direct peptide–major histocompatibility complex (MHC) binding studies in Api m 1 by direct binding of peptides to purified MHC class II molecules. Four dominant peptides were identified; three of these represented similar regions of the molecule to those employed previously.[56] These four peptides were evaluated in a controlled, open-label, single-blind study in subjects with mild bee venom allergy.[57] Treatment was well tolerated, with no allergic reactions observed. Proliferation of T cells to purified allergen and whole bee venom was significantly reduced after therapy. 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. Cutaneous late-phase reactions to challenge with whole bee venom or Api m 1 were significantly reduced. Allergen-specific IgG and IgE levels were measured, revealing a significant, transient increase in allergen-specific IgG and IgG4 following peptide immunotherapy.

RUSH desensitization was employed to treat bee venom–allergic subjects using three synthetic polypeptides spanning the whole Api m 1 molecule.[58] Patients received approximately 250 μg of each peptide in incremental doses at 30-minute intervals starting with 0.1 μg. Maintenance injections of 100 μg (in some cases 300 μg) were given on days 4, 7, 14, 42, and 70. T-cell proliferation increased transiently 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. Skin sensitivity to intradermal allergen challenge did not change significantly. Peptide therapy was generally well tolerated. However, local and disseminated erythema with occasional hand palm pruritus was observed in two subjects at higher doses.

In conclusion, peptide immunotherapy has been shown to improve clinical outcomes and surrogate markers in a variety of studies. Treatment appears to be associated with the induction of IL-10 and a population of allergen-specific regulatory or suppressor T cells. Numerous studies in both allergic and autoimmune diseases support the potential of this approach. However, significant issues still need to be addressed, including whether delivery of T-cell epitopes without competent B-cell epitopes will be sufficient to provide efficacy equivalent to conventional, whole-allergen immunotherapy. Short peptides (less than 20 amino acids) appear to have markedly reduced ability to cross-link allergen-specific IgE and are less allergenic than the whole molecule. However, delivery of high doses of peptide may activate memory effector T cells, resulting in T cell–mediated events such as isolated late asthmatic reactions. Recent data suggest that such reactions can be avoided with lower peptide doses, which are still capable of inducing tolerance. Care must be taken in peptide selection to ensure full population coverage based on the MHC-binding characteristics of the peptides. In practice, one peptide can contain several overlapping T-cell epitopes with affinity for a range of MHC molecules. Further clinical studies are required with preparations of short peptides derived from the sequences of other allergens.

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