Update review

Update on the current status of peptide immunotherapy

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The use of synthetic peptide fragments of allergen molecules holds promise for the delivery of effective immunotherapy without IgE-mediated adverse events. Early studies were associated with frequent induction of allergic symptoms after treatment, mostly related to activation of allergen-specific effector T cells with high doses of peptides. More recently, low doses of peptides have been shown to modify clinical and laboratory surrogates without inducing adverse events. Studies are ongoing to define the optimal dose, dose interval, and route of administration. Current results indicate that treatment with peptides modulates the immune response by reducing T_H2 responses to allergen and increasing IL-10 production and the activity of allergen-specific regulatory T cells. Further studies are required in larger numbers of subjects and with peptides derived from a variety of allergens. (J Allergy Clin Immunol 2007;119:906-9.)

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Strategies to improve the safety of allergen immunotherapy have traditionally focused on reducing the allergenicity of the preparation administered to the patient. The use of peptide fragments, corresponding to T-cell epitopes, to induce immunologic tolerance has been reported in experimental models of both allergic and autoimmune disease.[1] By virtue of their size and relative lack of secondary and tertiary structure, peptides display reduced ability to cross-link allergen-specific IgE and activate IgE receptor–bearing cells. However, retention of T-cell epitopes allows targeting of allergen-specific T cells for the induction of tolerance. Translation of this approach to the clinic is ongoing, with a number of small clinical studies having been performed over the last decade. At present, 3 allergens have been targeted with this approach (Table I): the cat allergen Fel d 1, the bee venom allergen Api m 1 (phospholipase A_2), and the ragweed allergen Amb a 1 (clinical studies of the last have not been reported). Considerable objective evidence has accumulated demonstrating modulation of allergen-specific immune responses after peptide immunotherapy. Evidence supporting clinical benefit has been slower to accumulate, and definitive studies demonstrating reduced symptom scores and medication use combined with improvements in tolerability are still required.

The earliest clinical studies employed a combination of 2 peptides (Allervax Cat, ImmuLogic Corp., Waltham, Mass) from Fel d 1, administered subcutaneously in a variety of dosing regimens. In the first of these to be reported, peptides were administered at weekly intervals and in 3 dose cohorts (7.5 μg, 75 μg, and 750 μg).[2] After allergen exposure in a cat room, lung and nasal symptom scores were significantly improved in the higher dose cohorts. Despite the use of peptides that had been screened for reduced activity in basophil histamine release assays, numerous adverse events were documented occurring minutes to hours after peptide administration. Some adverse events may have been a result of the relatively long peptides used that may have harbored residual IgE reactivity; however, the majority of events probably occurred as a result of activation of allergen-specific effector T cells resulting in late asthmatic reactions.[3] The same peptides were evaluated in inhaled allergen challenge studies. A significant increase in PD_{20} was observed within cohorts receiving higher dose (total dose between 150 μg and 4500 μg) regimens, but this failed to achieve statistical significance when compared with placebo.[4]

In a double-blind, placebo-controlled parallel group study, peptides or placebo were administered by weekly subcutaneous injection (4 × 250 μg) to 42 subjects with cat-allergic rhinitis and/or asthma.[5] Treatment was associated with adverse events, primarily late-onset symptoms of rhinitis, asthma, and pruritus. No significant changes were observed in the primary outcome measure (change in wheal and flare reaction) or in late-phase skin responses to allergen challenge. PBMC cytokine secretion profiles did not differ between peptide-treated and placebo-treated subjects.

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TABLE I. Clinical studies of peptide immunotherapy

First author	Allergen	No. of peptides and length	Total dose	Route	No. of subjects	Major outcome measures	Reference
Cat allergy							
Norman	Fel d 1	2 × 27	30-3000 μg	Subcutaneous	95	Improved symptom scores after cat room challenge	2
Simons	Fel d 1	2 × 27	1000 μg	Subcutaneous	42	No change in cutaneous allergen challenge	5
Pène	Fel d 1	2 × 27	15-4500 μg	Subcutaneous	31	Improved allergen PD_{20} compared with baseline	4
Maguire	Fel d 1	2 × 27	6000 μg	Subcutaneous	133	Improved FEV_1 in subjects with reduced baseline FEV_1	6
Oldfield	Fel d 1	12 × 16/17	5 μg	Intradermal	24	Reduced cutaneous late-phase reaction after allergen challenge	7
Oldfield	Fel d 1	12 × 16/17	90 μg	Intradermal	24	Reduced cutaneous early and late-phase reaction after allergen challenge, T_H1/T_H2 cytokines reduced, IL-10 increased	8
Alexander	Fel d 1	11 × 16/17	40.1 μg	Intradermal	8	Reduced airway hyperreactivity and cutaneous late-phase reaction after allergen challenge, increase in $CD4^+IFN\text{-}\gamma^+$ cells in skin after allergen challenge	9
Alexander	Fel d 1	12 × 16/17	131-341 μg	Intradermal	28	Improved nasal symptoms and late asthmatic reaction after allergen challenge	11
Bee venom allergy							
Müller	Api m 1	3: 11, 12, 18	397.1 μg	Subcutaneous	5	Improved tolerance of skin allergen challenge and partial protection from live sting challenge	13
Fellrath	Api m 1	3: 45, 53, 60	751.1-1551.1 μg	Subcutaneous	16	Increased allergen-specific IgG_4, increased IFN-γ and IL-10 in PBMCs	14
Tarzi	Api m 1	4 × 18	431.1 μg	Intradermal	12	Reduced cutaneous early and late-phase reaction after allergen challenge, T_H1/T_H2 cytokines reduced, IL-10 increased, transiently increased IgG_4	15

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Treatment with Allervax Cat was also associated with a significant clinical improvement in pulmonary function (FEV_1) in individuals with reduced baseline FEV_1.[6] Furthermore, a significant improvement in the subjective ability to tolerate cats was also observed through a visual analogue evaluation. Importantly, subjects recruited to this study had moderate to severe disease, with many demonstrating high IgE levels. Approximately 30% had failed previous whole allergen immunotherapy, and more than 40% of the group had asthma. Adverse events were common and consisted of occasional IgE-mediated acute reactions and more frequent late-onset symptoms of asthma. Approximately 20% of peptide-treated subjects developed late-onset symptoms after the first peptide administration, but the prevalence of these reactions decreased as dosing continued, indicating the induction of immunologic tolerance.

The incidence of late adverse events was related to both peptide dose and the severity of disease in the study group. Subjects with asthma appeared more likely to develop late-onset symptoms and particularly at higher peptide doses.[2,6] A weak relationship was also established between levels of allergen-specific serum IgE and the propensity to develop late reactions.[6] Some subjects developed *de novo* IgE specific for the treatment peptides, and this was associated in some cases with acute adverse events.[6]

Thus, early clinical experience established that peptide immunotherapy could modify immunologic markers, clinical surrogates, and subjective clinical outcomes in some studies. The development of peptide-specific IgE and the association of late adverse events with peptide dose and disease severity indicated that improved safety and tolerability may be achieved by using smaller peptides with reduced ability to elicit and interact with IgE (the Allervax Cat peptides were 27 amino acids in length, whereas an average CD4 T-cell epitope would be approximately 14 amino acids). Furthermore, evaluation of lower peptide doses in subjects with less severe disease was indicated.

A number of clinical studies have been performed more recently by using mixtures of shorter peptides from Fel d 1.[3,7-12] Peptides were administered intradermally to subjects with cat-allergic asthma. Dose-dependent

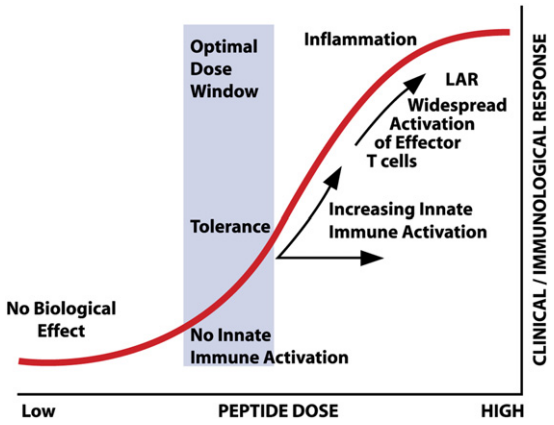

FIG 1. The effect of dose in peptide immunotherapy. In the *optimal dose window,* peptide is delivered in sufficient quantities to modify T-cell responses in a noninflammatory context leading to tolerance. Lower doses are insufficient to induce a clinical response. Higher doses are recognized in an inflammatory context leading to activation of effector T cells and adverse events. *LAR,* Late asthmatic reaction.

induction of isolated late asthmatic reactions was observed after a single injection of a mixture of 12 peptides, but this was associated with subsequent tolerance to further challenge that lasted several months. Indeed, peptide injection, whether associated with the induction of late reactions or not, resulted in significantly reduced cutaneous late-phase reaction to intradermal allergen challenge. Furthermore, evaluation of PBMC responses to allergen *in vitro* demonstrated reductions in both T_H2 cytokines and IFN-γ.[7]

The same 12-peptide mixture was subsequently administered in divided, incremental doses over a 2-week period in a double-blind, placebo-controlled study. Twenty-four subjects with cat-allergic asthma were subjected to cutaneous allergen challenge, inhaled methacholine PC_{20}, and inhaled allergen PD_{20}.[8] A total of 90 μg of each peptide was administered. Treatment was associated with a significant reduction in both early and late-phase cutaneous reactions to intradermal challenge with allergen compared with placebo. Reduced allergen-specific proliferative responses and T_H1 and T_H2 cytokine production were observed in the active treatment group. Production of IL-10 from PBMCs increased. On the basis of visual analogue evaluation, treated individuals were significantly less sensitive to cat exposure after therapy, although perhaps because of small numbers, this did not achieve significance compared with the placebo group. No improvement in PD_{20} or PC_{20} was observed.

In a related (open) study, a significant improvement in PC_{20} was observed with a lower dose regimen (total dose, 41.1 μg) with 2-week dose intervals.[9] The magnitude of the cutaneous late-phase reaction was significantly reduced after allergen challenge, confirming previous findings. Treatment was associated with an accumulation of both $CD25^+$ cells and CD4+/IFN-γ^+ cells in allergen challenge skin sites compared with placebo challenge. Expression of TGF-β mRNA but not IL-10 was increased after therapy.

In an attempt to determine optimal dosing regimens, 2 overlapping studies evaluated higher peptide doses (approximately 300 μg of each of 12 peptides from Fel d 1).[11] Subjects with cat-allergic asthma 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. Subjects with dual responses were recruited into a small double-blind, placebo-controlled (n = 16; 8 active, 8 placebo) study, whereas those displaying single early responses (n = 12) were recruited into an open study. Both groups received intradermal peptide injections in an incremental fashion (1 to 100 μg per injection). In common with earlier studies, the magnitude of the cutaneous late-phase reaction to intradermal challenge with allergen extract was significantly attenuated. Similarly, the late asthmatic reaction after allergen inhalational challenge was also attenuated (in the group who experienced late-phase responses). In the open study, there was a significant reduction in outcome scores (sneezing, weight of nasal secretions, and nasal blockage) after nasal allergen challenge, together with significant improvements in some rhinitis quality of life questionnaire fields. No change was observed in allergen PD_{20} or histamine PC_{20}.

Studies evaluating the ability of peptide treatment to modulate regulatory T-cell function have shown no change in the function of blood $CD4^+CD25^+$ regulatory T cells[10] but revealed the induction of a population of adaptive, allergen-specific $CD4^+$ T cells with functional regulatory capacity.[12]

BEE VENOM ALLERGY

Three studies of immunotherapy with peptides derived from the major allergen Api m 1 (phospholipase A_2) have been reported. In an open study, 5 subjects with bee venom allergy received divided incremental doses of a mixture of 3 peptides at weekly intervals.[13] Patients received 397.1 μg of each of 3 peptides subcutaneously. Patients receiving conventional bee venom immunotherapy were used as controls. After treatment, subcutaneous allergen challenge was tolerated without systemic allergic symptoms. Three of 5 subjects also tolerated a wild bee sting challenge without reaction. The remaining 2 subjects developed mild systemic allergic reactions. Allergen challenge was associated with a marked increase in serum levels of allergen-specific serum IgG_4.

After encouraging results in a murine model of bee venom sensitivity, subjects with bee venom allergy were treated with 3 synthetic polypeptides (long synthetic peptides ranging in length from 45 to 60 residues) covering the whole Api m 1 molecule according to a RUSH protocol.[14] Subjects initially received approximately 250 μg in incremental divided doses at 30 minute intervals. As many as 5 maintenance injections were subsequently given with total doses reaching approximately 750 to 1150 μg. Some mild adverse events were reported. IFN-γ, IL-10, and allergen-specific IgG_4 levels increased.

Peptide-specific IgE was induced in some patients. In contrast with studies using shorter peptides, no significant change in skin sensitivity to intradermal allergen challenge was observed.

Most recently, four 18–amino acid peptides with high affinity for commonly expressed MHC class II molecules were administered to individuals with bee venom allergy with mild disease in a controlled, open-label, single-blind study.[15] A total dose of 431.1 μg of each of the 4 peptides was administered to 12 individuals, with untreated subjects matched for disease severity acting as controls. No adverse events were observed. In common with studies using cat allergen peptides, treatment with bee venom peptides was associated with reduced PBMC proliferative and T_H1/T_H2 cytokine responses. A concomitant increase in IL-10 production was observed. Late-phase skin reactions to allergen were significantly reduced. A transient but significant increase in allergen-specific IgG_4 and IgG was also observed.

In summary, translation of peptide immunotherapy from encouraging murine models and early human studies continues. Optimal dose schedules have yet to be established, but early indications are that unlike whole allergen immunotherapy, higher doses may not always be more effective (Fig 1). Subjects with more severe disease may experience treatment-related adverse events at higher peptide doses. Low doses that do not elicit adverse events are able to induce immunologic tolerance and to modify surrogate clinical markers. Adverse events appear to be related to residual IgE reactivity in larger peptides and to activation of allergen-specific effector T cells at high peptide doses. Therapeutic effects appear to be associated with the induction of allergen-specific regulatory T cells and can be achieved at doses low enough to avoid adverse events. Further studies are required to define optimal dose, dose interval, and route of administration.

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