

Peptide-based vaccination: where do we stand?

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Purpose of review

Allergen-specific immunotherapy represents the only causative approach towards allergy treatment. Specific immunotherapy can, however, include allergic reactions and occasionally life-threatening anaphylaxis. Peptides have been evaluated as a potential therapeutic approach in atopic allergic disease because they have the potential to inhibit T-cell function but not induce anaphylaxis.

Recent findings

Data from early clinical trials of peptide vaccination revealed that therapy was associated with a modest improvement in allergic disease, and was accompanied by a high frequency of adverse reactions. More recent studies have demonstrated improved clinical outcomes, improved safety, and have defined the mechanisms of adverse events observed in earlier studies. Mechanisms of peptide vaccination include the hyporesponsiveness of allergen-specific responses and the induction of regulatory T cells and cytokines. Novel peptide design has allowed the generation of fragments that contain T-cell stimulatory epitopes, lack B cell epitopes, and can induce protective IgG responses in both mice and humans. Other approaches have focused on hypoallergenic B-cell epitopes that induce inhibitory IgG antibodies. Peptides that specifically induce regulatory cytokine production would also enhance peptide vaccines. Several recent studies have described immunodominant epitopes from major allergens that may form candidate peptides for use in peptide vaccination.

Summary

The manipulation of peptide epitopes may provide a strategy for the rational design of peptide allergy vaccines further improving safety and efficacy.

Keywords

allergy, epitope, immunotherapy, peptide, vaccine

Abbreviations

LAR	late asthmatic reaction
PBMC	peripheral blood mononuclear cell
TGF-β	transforming growth factor beta
Th	T helper

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Introduction

Specific allergen immunotherapy, or allergy vaccination, for the treatment of IgE-mediated disease was first described almost 100 years ago [1], and is based on the application of small but increasing doses of allergen to which the patient is sensitized. Historically, relatively crude allergen extracts have been used for specific allergen immunotherapy, but for certain allergens, such as peanut and latex, these are associated with a high risk of life-threatening anaphylaxis [2]. More recently, attention has focused on recombinant allergens and peptides that display reduced IgE binding while retaining T-cell epitopes, thus reducing the possibility of adverse reactions. Here we describe the clinical trials using peptide vaccination to treat allergic disease and the corresponding in-vitro experiments designed to delineate the underlying mechanisms. We also report on recent data describing the induction of regulatory cells after cat peptide vaccination, which may account for the decreased allergen-specific T-cell responses observed after treatment. Conventional specific allergen immunotherapy is associated with the induction of 'protective' IgG antibodies. Recent data demonstrate that the administration of peptides representing either T or B-cell epitopes are also able to induce these inhibitory IgG antibodies. Allergic sensitization to common allergens is often associated with cross-sensitization to other allergens that share common T-cell epitopes. Recent reports have suggested that the induction of hypoallergenicity to the major allergen may also reduce responses to cross-reactive allergens. Finally, we describe the characterization of various T-cell epitopes from common allergens that have the potential to be utilized in peptide immunotherapy.

Conventional immunotherapy

Specific allergen injection immunotherapy is a highly effective treatment for IgE-mediated allergic diseases including venom anaphylaxis and seasonal rhinoconjunctivitis and asthma [2,3]. Immunotherapy may confer long-term benefit for at least 3 years after discontinuation [4,5], prevent the onset of new sensitizations, and reduce the

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progression of seasonal rhinitis to asthma in children [6,7]. Immunotherapy has generally been administered by the subcutaneous injection of natural allergen extract, although recently sublingual administration has become popular as this form of therapy has a good safety profile and does not require injections. For pollen immunotherapy, vaccines are delivered in gradually increasing doses during an up dosing phase until a maintenance dose is reached, and subsequent doses are administered every 1–2 months. Allergen immunotherapy has been shown to be clinically effective [8,9].

The mechanisms associated with immunotherapy are thought to involve both cellular and humoral immune responses [10]. These include a modest reduction in allergen-specific IgE and increases in allergen-specific IgG antibodies, particularly of the IgG4 isotype [11–13]. Effector cells are reduced at the site of allergic inflammation, including a reduction in the numbers of mast cells, eosinophils, basophils and the release in mediator secretion [14,15]. The modulation of T-cell responses after immunotherapy are thought to induce immune deviation towards a protective T helper (Th) type 1 response [16,17] or the induction of regulatory T cells producing IL-10 and transforming growth factor beta (TGF- β) [18–21].

Although specific immunotherapy is a highly effective form of treatment for allergic disease, one major drawback of this treatment is the observed adverse reactions to relatively high doses of allergen that can sometimes lead to anaphylaxis. Therefore, other approaches to allergy vaccination have been investigated that aim to avoid the cross-linking of IgE. Peptides have the potential to inhibit T-cell function but not induce anaphylaxis, because of the loss of three-dimensional conformational determinants, and therefore provide a suitable form of treatment with reduced capacity to induce anaphylaxis.

Clinical trials of cat peptide vaccines

The most extensive clinical studies have involved individuals with allergic disease to cats. In 1996, Norman and colleagues [22] investigated the therapeutic effects of treating cat-allergic individuals with two polypeptides derived from the cat allergen, Fel d 1. Using three different doses of peptides, treatment was administered in four injections to 95 subjects. The high-dose group (75 μ g) reported a statistically significant decrease in symptom scores although treatment was also associated with a high incidence of adverse events. A second study by Pene and colleagues [23] revealed that treatment with higher peptide doses could promote a significant reduction in bronchial reactivity compared with baseline measurements, but the effects did not reach significance when compared with the placebo group. A larger multi-centre trial administered eight injections

of 750 μ g of the same peptides to cat allergic individuals [24]. Some improvements in pulmonary function were observed, but these were only evident in a subset of subjects and only at a single timepoint post therapy. Another study utilized 12 peptides, which encompass most of the T-cell epitopes within Fel d 1 in a double-blind placebo controlled trial [25]. Twenty-four cat-allergic individuals were treated with a total of 90 μ g of each of the 12 peptides or with placebo alone. Significant reductions in late-phase responses in intradermal challenge with purified Fel d 1 occurred both 4–8 weeks after treatment (first follow-up) and at 3–9 months (second follow-up) compared with placebo. Reductions in early phase responses were reduced at the second follow-up after initial treatment.

Mechanistically, such studies have reported reduced IL-4 production using either T-cell lines [26] or peripheral blood mononuclear cell (PBMC) cultures after allergen stimulation [27], although others have failed to observe any changes in PBMC cytokine secretion profiles [28]. Studies using multiple peptides have shown decreased proliferative responses and the reduced production of IFN- γ , IL-13 and IL-4 after allergen stimulation of PBMCs [25]. These responses were accompanied by significant increases in allergen-stimulated IL-10 production.

In summary, whereas such studies generally reported modest improvements in clinical and surrogate outcome measures, treatment was associated with a high frequency of adverse reactions. It is possible that the peptides had formed homo and heterodimers, which in this form could crosslink adjacent IgE molecules on the surface of mast cells or basophils. This may account for any early adverse reactions caused by peptide injection, but treatment was also associated with late reactions such as chest tightness and wheeze.

Investigation of the mechanisms causing adverse reactions

Late asthmatic reactions (LARs) after peptide therapy (a delayed and prolonged reduction in forced expiratory flow rate, in the absence of acute bronchoconstriction) have been well characterized by various studies [29]. Either the intradermal or intranasal delivery of peptides representing the T-cell epitopes of Fel d 1 to individuals with mild to moderate disease could induce a delayed, prolonged reduction in FEV1 representing LARs [30,31]. Interestingly, if individuals experiencing LAR after peptide challenge were given a second dose of peptide via the same route, a reduced or absent response was observed. This hyporesponsiveness was shown to be transient, as LARs returned to baseline levels if the rechallenge was administered 3–6 months after the initial tolerance induction [32]. Investigation of a surrogate

marker of systemic reactivity to allergen, the cutaneous late phase response, again revealed reduced responsiveness to allergen after an initial peptide challenge. This reduction was also apparent in individuals who did not experience LARs, suggesting that the mechanisms underlying the induction of LARs were secondary to the induction of hyporesponsiveness. This mechanism is thought to be T-cell mediated as (1) LAR occurs a number of hours after the initial challenge; (2) increased CD4⁺ T-cell numbers are observed in the bronchial mucosa post challenge; (3) individuals experiencing LARs preferentially expressed major histocompatibility complex alleles capable of binding to one or more of the administered peptides.

Clinical trials for bee venom allergy

Another allergic disease that has been investigated for a trial of peptide immunotherapy is bee venom allergy. The major allergen found in bee venom is phospholipase A2 (Api m 1). In a study by Muller *et al.* [33] five bee venom-allergic patients received divided incremental doses of three peptides derived from Api m 1, to a total dose of almost 400 µg. After therapy, subjects were able to tolerate challenge with allergen and three out of five tolerated a live bee sting challenge without reaction. After a similar protocol, but using peptides that were selected on the basis of direct major histocompatibility complex binding characteristics [34], Tarzi and colleagues (unpublished observations) performed a controlled, open-label study of peptide immunotherapy in individuals with mild (no history of anaphylaxis) bee venom allergy. Peptide therapy was well tolerated and resulted in a significant reduction in the magnitude of the late-phase cutaneous reaction to both whole bee venom and purified Api m 1 after challenge. Both studies demonstrated that stimulation of PBMC with whole allergen extract resulted in reduced proliferative responses and a reduction in both Th1 and Th2 cytokine production *in vitro*. The latter study also reported that a significant increase in levels of IL-10 was observed after therapy.

Induction of regulatory T cells by peptide immunotherapy

Conventional allergen-specific immunotherapy is associated with increases in CD4⁺CD25⁺ regulatory T cells producing IL-10 or TGF-β. Three recent reports have investigated these cells in the context of cat peptide immunotherapy. The first study by Alexander *et al.* [35] was an open study of eight cat-allergic individuals who received cat peptide immunotherapy using an 11-peptide mixture. Clinically, all subjects showed an improvement in non-specific bronchial hyperreactivity (increase in histamine PC₂₀) levels and significant decreases in cutaneous late-phase reactions. Immunohistochemistry of biopsy tissue obtained after skin allergen challenge showed a significant increase in the number of

CD25-positive cells compared with placebo challenge after peptide immunotherapy. No increases in IL-10-positive cells were observed in the skin after treatment although the number of CD4/IFN-γ-positive cells increased, suggesting that the recruitment of Th1 cells to the skin may be an important mechanism. In addition, higher levels of TGF-β messenger RNA were observed in the skin after peptide therapy.

A second report aimed at investigating the effect of peptide immunotherapy on peripheral blood CD4 cell responses and CD4⁺CD25⁺ suppression of allergen-stimulated cultures in a double-blind, placebo-controlled trial [36]. Allergen stimulation of cells obtained post-treatment revealed a reduction in proliferative responses and IL-13 production compared with baseline samples. Suppressing activity of CD4⁺CD25⁺ cells was measured by mixing this cell population with CD4⁺CD25⁻ cells from the same donor. Using similar methodology, other studies have shown that the suppressive activity of allergic donors is impaired compared with normal donors, and it was hypothesized that immunotherapy could restore this activity [37]. Peptide immunotherapy did not, however, alter the suppressive activity of these cells and allergen stimulation of post-immunotherapy samples did not induce IL-10 production in this study.

A third report from the same group utilized some novel and elegant techniques to study potential regulatory differences in CD4 T-cell populations before and after cat peptide immunotherapy (i.e. beyond the remit of CD4⁺CD25⁺ cells) [38^{••}]. Results showed that antigen-specific proliferative responses of memory T cells were reduced after peptide immunotherapy compared with baseline samples. Further experiments demonstrated that CD4 cells isolated after peptide immunotherapy suppress the proliferative response of baseline CD4-negative cells in co-culture experiments. These data suggest that peptide immunotherapy can induce a population of CD4 T cells with suppressive/regulatory activity.

Inhibitory antibodies induced by immunotherapy

One proposed mechanism of successful immunotherapy is the induction of inhibitory or blocking antibodies [39]. A general observation is that during specific allergen immunotherapy, increases in allergen-specific IgG1 and IgG4 antibody concentrations occur that correlate with the clinical outcome [13,19,21,40]. The mechanisms by which allergen-specific IgG antibodies are thought to exert their effects is either by blocking basophil histamine release [41,42] or by the competitive inhibition of allergen-IgE complexes by IgG, which prevents complexes binding to the low-affinity IgE receptor, CD23, and subsequent antigen presentation [43,44]. These

effects are likely to be caused by the increased serum concentration of these antibodies and not antibody affinity maturation [45,46].

As peptides contain little or no secondary or tertiary structure it is possible that they cannot induce specific antibodies against wild-type allergen as these generally recognize determinants formed on complete tertiary folded molecules. Some studies of peptide immunotherapy have failed to report changes in IgE or IgG antibodies after treatment unless whole allergen challenge is also provided [33]. In contrast, using a similar selection of phospholipase A2 peptides, Tarzi and colleagues (unpublished data) demonstrated a transient but significant increase in phospholipase A2 and bee venom-specific IgG and IgG4. Recent data also suggest that treatment with peptides can induce inhibitory IgG antibodies without the generation of IgE antibodies. For example, one study has utilized genetic engineering to generate two non-overlapping fragments of the Bet v 1 antigen, which are approximately 75 amino acids in length. Whereas fragments show negligible binding of IgE from birch pollen-allergic individuals (thus avoiding the potential of IgE crosslinking) they still retain their T-cell-reactive epitopes [47]. Interestingly, mice immunized with these fragments were able to produce IgG antibodies, which inhibited the binding of allergic patients' IgE to the whole allergen. Both these fragments represent unfolded molecules [48], and therefore it is unlikely that they could induce antibodies to conformational (discontinuous) epitopes. This ability to block IgE binding may be explained by the antibody binding in close proximity to the IgE binding epitopes thus causing steric hindrance (the physical presence of one molecule blocking access of another), or second, the IgG antibodies may recognize continuous portions within discontinuous IgE epitopes. Therefore, it is possible to generate blocking antibodies against wild-type antigen using peptides with no secondary structure. The Bet v 1 fragments were subsequently evaluated in a double-blind placebo-controlled trial of birch pollen immunotherapy in humans [49[•]]. Results showed that individuals treated with these fragments showed a reduction in cutaneous sensitivity as well as an improvement of symptoms. Interestingly, vaccination with the fragments induced highly significant increases in allergen-specific IgG antibody levels after treatment. These IgG molecules were shown to be functionally active *in vitro* using a histamine release assay.

An alternative approach to the identification of T-cell epitopes from allergens, in order to induce tolerance or immune deviation, is to aim solely to induce protective antibodies. Focke and colleagues [50] identified peptides derived from the major grass pollen allergen, Phl p 1, which primarily represent B-cell epitopes. These peptides were shown to lack any secondary and tertiary

structure and failed to induce a positive skin-prick test in allergic individuals or induce basophil histamine release. When used as an immunogen in mice and rabbits, however, these peptides induced protective antigen-specific IgG antibodies, which could inhibit the binding of grass-pollen allergic patients' IgE binding to Phl p 1. In addition, this group has recently identified similar peptides from the major birch pollen allergen that have similar properties [51[•]]. This group suggested that the use of peptides that induce blocking antibodies are an important consideration in vaccine design.

Use of peptides containing cross-reactive epitopes

Individuals with allergies to airborne allergens often also experience hypersensitivity reactions to certain foods as a result of cross-reactive IgE antibodies. For example, up to 70% of individuals with birch pollen allergy exhibit this so-called 'oral allergy syndrome' on contact with raw vegetables or fruits such as celery and apple [52]. A recent report [53[•]] identified the dominant T-cell epitope of Bet v 1 and investigated the cellular cross-reactivity with homologous food antigens. The immunodominant epitope of Bet v 1 was recognized by 61% of allergic individuals, and T cells recognizing this region also evoked cellular responses to a variety of food antigens containing homologous regions. If such cross-reactivity also occurs *in vivo* the exposure of cross-reactive food allergens might promote birch-specific T-cell responses out of the pollen season. If cross-reactive peptides were used for the immunotherapeutic treatment of birch-sensitive individuals, however, it is plausible that reduced sensitivity to both birch allergen and to the cross-reactive food antigen may occur. Using genetically modified allergen fragments of birch pollen, as discussed earlier, seven out of 25 actively treated individuals with oral allergy syndrome reported an improvement in symptoms compared with one out of 18 subjects in the placebo group [49^{••}].

Characterization of peptide epitopes of other allergens: recent advances

In order to develop a T-cell-targeted vaccine for use in allergy vaccination it is crucial to identify dominant T-cell epitopes of the appropriate allergen. The vast majority of research into candidate peptide epitopes has concentrated on cat, grass pollen, birch and bee venom allergens. A number of recent reports have, however, identified peptide epitopes from other major allergens.

Mugwort pollen allergy is frequently encountered in central Europe, and the major allergen in mugwort pollen, Art v 1, is recognized by almost all sensitive individuals. Unlike other allergens that typically contain multiple T-cell epitopes, Art v 1 has only one immunodominant

epitope, Art v 125–36 [54[•]]. This peptide would be a good candidate for use in peptide immunotherapy as it is recognized by 96% of Art v 1-reactive patients, which means that most patients could be treated with this single peptide. Also, it does not contain any IgE binding domains, the epitope is common to all natural forms of Art v 1, and the recombinant isoform of rArt v 1, Art v 125–36, can elicit similar T-cell responses *in vitro* compared with the natural allergen.

A recent study by Glaspole *et al.* [55] identified and characterized the T-cell epitopes of the major peanut allergen, Ara h 2. Using a set of overlapping peptide fragments spanning the entire sequence of Ara h 2, two highly immunogenic T-cell-reactive regions have been identified. These clusters of peptide were associated with increased proliferative responses and induced elevated IL-5 production.

Drew and colleagues [56[•]] investigated the immunodominant T-cell epitopes of Hev b 6.01, the major allergen of natural rubber latex, which is an important cause of latex sensitization in latex glove users. Using site-directed mutagenesis, mutant forms of the immunodominant peptides were generated that showed ablated allergenicity while retaining their latex-specific T-cell reactivity. The authors commented that such mutants could be used in conjunction with other altered peptide epitopes from latex allergens [57] to form a hypoallergenic desensitizing vaccine.

Future peptide vaccine design

The selection of the appropriate ligands for use in peptide immunotherapy remains a difficult choice. Ideally, peptides must have: (1) the ability to bind to diverse HLA types; (2) the ability to induce IFN- γ or IL-10; and (3) the ability to induce the generation of inhibitory IgG antibodies. These ideals may underlie the protective effect of high-dose natural exposure to cat allergens, as reported by Carneiro and colleagues [58[•]]. Cat-allergic individuals, although having high allergen-specific IgE levels, also produce IgG4, which appeared to protect from disease. This was described as a 'modified Th2 response'. Analysis of particular peptides from Fel d 1 suggested the preferential induction of IL-10. Such peptides could be included in future trials of cat peptide vaccination to enhance vaccine efficiency.

Conclusion

Numerous strategies to reduce the allergenicity of therapeutic preparations for the treatment of allergic diseases are being developed. Short, synthetic peptides representing T-cell epitopes of the major allergens showed substantially reduced ability to crosslink allergen-specific IgE. In a number of clinical studies, peptide treatment has been shown to modify surrogate markers of allergen

exposure, such as cutaneous responses to intradermal allergen challenge, to improve bronchial hyperreactivity (some studies), to improve symptom scores after nasal allergen challenge, and to improve patients' ability to tolerate natural allergen exposure. Mechanistic studies indicate that peptide therapy induces both immune deviation (Th2 to Th1 response) and regulatory T cells capable of suppressing allergen-specific immune responses. Few data are available on the effects of peptide therapy on humoral immune responses. In particular, there appears to be substantially less allergen-specific IgG induced during peptide therapy (compared with conventional allergen extract immunotherapy). The reduced size of peptides and the relative lack of conformational determinants are associated with a reduced risk of IgE-mediated adverse events such as anaphylaxis. It remains to be seen whether the clinical response to peptide immunotherapy will be of equivalent efficacy to conventional treatment. Further peptide therapy studies and comparisons with existing therapy are eagerly anticipated.

References and recommended reading

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 581).

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