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Peptide-Based Immunotherapy: New Developments

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Introduction

Allergic diseases such as asthma, rhinoconjunctivitis and eczema are becoming increasingly common. The increased incidence may be related to modern lifestyle. A number of pharmacological approaches are available to alleviate symptoms, including anti-histamines, topical corticosteroids and inhaled bronchodilators. However, many patients with moderate to severe disease experience only partial to poor symptom control. Furthermore, they are often unable to adopt standard allergen avoidance techniques, as the aeroallergens responsible for disease are widespread in the environment.

At present the only disease-modifying approach for patients refractory to conventional pharmacotherapy is specific or whole allergen immunotherapy (SIT). This involves the administration, usually by subcutaneous (s.c.) injection, of increasing doses of allergen extracts [1-3]. SIT is associated with a significant risk of IgE-mediated adverse events including systemic anaphylaxis. Other difficulties encountered with SIT include the poor standardization of allergen extracts and the lengthy duration of treatment that can lead to a failure in patient compliance. Thus, efforts are being focused on the development of other strategies to treat pharmacologically resistant disease.

The role of T lymphocytes in allergic responses has been studied extensively, identifying them as potential targets for therapeutic intervention. CD4⁺ T-helper-2 (Th2)-type cells are increased at tissue sites in allergic diseases and asthma [4-6], accumulate at sites of allergen challenge [7-9], and correlate with symptoms [10]. Th2 cells are capable of orchestrating the allergic response as cytokines produced by these cells, including interleukin (IL)-4 and IL-5, are relevant in promoting immunoglobulin class switching to IgE and can initiate and stimulate the differentiation, survival, and chemotaxis of eosinophils and other effector cells that release mediators in target tissues [11]. Thus, the ability to downregulate Th2-type cell function represents an important therapeutic

strategy. Peptide immunotherapy is an approach that targets CD4⁺ T-cells by using allergen-derived peptides containing short linear T-cell epitopes to induce tolerance. The principle has been established in animal models and the reduced ability of short peptide epitopes to cross-link surface-bound IgE may provide an attractive alternative to SIT in humans.

Peptide-induced hyporesponsiveness in vitro

In vitro experiments have demonstrated that different concentrations of peptides can induce activation or hyporesponsiveness depending on dose. Lamb and colleagues [12] employed high doses (50 mg) of peptide to render human T-cells non-responsive *in vitro*. Influenza virus hemagglutinin-specific human Th0 T-cell clones were exposed to supraoptimal doses of peptide in the absence of antigen-presenting cells. Subsequent whole antigen challenge was characterized by antigen-specific T-cell hyporesponsiveness (anergy), which could be prevented or reversed by the addition of IL-2 [13]. Further studies using supraoptimal doses of peptides in human CD4⁺ T-cell clones reactive to house dust mite reproduced clonal anergy and showed that this state was accompanied by down-regulation of IL-2 and IL-4 and maintenance of IFN- γ secretion [14,15].

Investigation of T cell responses in vivo: Animal models

The principle that T cell peptide epitopes may be employed to induce antigen-specific hyporesponsiveness has been extensively investigated in rodent models of autoimmune disease and, more recently, in models of allergic sensitization.

Briner and colleagues established a murine model of cat allergy in which two peptides (IPC-1 and IPC-2), derived from chain 1 of Fel d 1, the major cat allergen, were administered [16]. Decreased IPC-2-specific production of IL-2, IL-4 and IFN- γ was demonstrated. In addition, antigen-specific T-cell proliferation was reduced. The effects of subcutaneous IPC-2 administration on animals already primed with Fel d 1 was also examined. Combined administration of IPC-1 and IPC-2 resulted in a decrease in the T-cell response (IL-2 production) to stimulation with the whole chain 1 protein.

Using the mucosal route, Hoyne and co-workers performed intranasal peptide challenge and demonstrated the induction of T-cell tolerance [17,18]. Peptides derived from the house dust mite allergen Der p 1, were not only able to prevent sensitization but could also inhibit T-cell responses in sensitized mice. Hyporesponsiveness was short-lived, since re-challenge at day 21 was able to elicit a further transient T-cell response that was subsequently down-regulated over time. These and other animal studies have demonstrated that antigen-specific hyporesponsiveness can be achieved through the administration of peptides.

Clinical Studies

Approximately 9% of the population with allergy are sensitive to house dust mite. The allergen is ubiquitous in the home and also in public places. Studies of allergen mapping and preclinical studies of peptide sequences, IPC-1 and

Norman and colleagues [19] conducted a double-blind, randomized, placebo-controlled study through a double-blind, randomized, placebo-controlled study. Weekly subcutaneous injections of IPC-1 and IPC-2 were administered to 12 patients in the highest dose group, six with a significant nasal congestion and six with no nasal congestion. IPC-1/IPC-2-specific IgG production and dosage was demonstrated. The allergen remained in the

In a further study, Norman and colleagues [20] conducted a double-blind, randomized, placebo-controlled study. Inhaled allergen challenge (FEV1) before and after treatment with IPC-1/IPC-2, ending therapy, post-challenge FEV1, treated and placebo groups. Significant improvement in FEV1 and release was significant. IFN- γ production.

In a randomized, double-blind, placebo-controlled study, patients received s.c. injections of IPC-1 and IPC-2. No change was seen in FEV1 after 24 weeks after treatment. Cytokine production and asthma, rhinitis and

Maguire and colleagues [21] conducted a double-blind, randomized, placebo-controlled study. Allergic patients (23) received IPC-1 or placebo twice weekly for a total of eight injections. Patients treated with 750 μ g of IPC-1 line FEV1, at a single dose, had a higher number of adverse events than the placebo group. In three patients with respiratory symptoms, successive doses.

Clinical Studies

Approximately 95% of individuals with a clinical history to cat (*Felis domesticus*) allergy are sensitive to one protein, Fel d 1, found in cat dander and saliva. The allergen is ubiquitous in the environment, present not only in the homes of cat owners but also in public places and is transported on clothing [19]. Cloning, sequencing, epitope mapping and preclinical studies resulted in the selection of two 27 amino acid sequences, IPC-1 and IPC-2 for evaluation in clinical trials [20].

Norman and colleagues evaluated the efficacy of Allervax[®]CAT (IPC-1/IPC-2) through a double-blind, placebo-controlled trial involving 95 individuals [21]. Four weekly subcutaneous injections of placebo or peptides at doses of 7.5 µg, 75 µg or 750 µg were administered to cat-allergic individuals. Clinical benefit was observed in the highest dose group, six weeks after the end of treatment. Peptide injections were associated with a significant number of early and late adverse events, including chest tightness, nasal congestion and flushing. In an associated study, a decrease in IL-4 production by IPC-1/IPC-2-specific T cell lines from subjects in the group that received the high dosage was demonstrated. However, proliferative responses to either peptide or whole allergen remained unchanged [22].

In a further study [23], the effect of treatment with IPC-1/IPC-2 was evaluated using inhaled allergen challenge (PD₂₀; provocative concentration causing a 20% reduction in FEV₁) before and after a variable cumulative dose of Allervax[®]CAT. Six weeks after ending therapy, post-treatment PD₂₀FEV₁ was not significantly different between the treated and placebo groups. However, in the middle and high dose groups, there was a significant improvement between baseline and post-treatment days. In addition, IL-4 release was significantly reduced in the high dose group. No change was observed in IFN-γ production.

In a randomized, double-blind, parallel-group study [24], forty cat-allergic subjects received s.c. injections of 250 µg of Allervax[®]CAT weekly for four consecutive weeks. No change was seen in either early- or late-phase skin reactivity to whole cat extract up to 24 weeks after the last injection. No significant change in cat antigen-specific cytokine production was observed. Frequent adverse events including symptoms of asthma, rhinitis and pruritus were reported.

Maguire and co-workers demonstrated modest improvements in some clinical outcome measures in a randomized, double-blind, placebo-controlled study of 133 cat-allergic patients [25]. Subjects received s.c. injections of either 75 µg or 750 µg peptides or placebo twice weekly for two weeks in two treatment phases, four months apart (a total of eight injections). Pulmonary function was improved in the group that were treated with 750 µg peptide per injection, but only in those subjects with reduced baseline FEV₁, at a single time-point three weeks post initial treatment phase. A large number of adverse events were recorded including the requirement for systemic epinephrine in three peptide-treated patients. The majority of adverse events were associated with respiratory symptoms, occurred a few hours after injection and declined with successive doses.

Peptide therapy for bee venom allergy

Müller and colleagues used an equimolar mixture of three T-cell epitopes of PLA₂, the major allergen of bee venom phospholipase A, to treat five patients who had experienced IgE-mediated systemic allergic reactions to bee stings [26]. Ten patients allergic to bee venom receiving SIT served as control subjects. Peptides were administered in an incremental dosing schedule starting at 0.1 µg and leading to three maintenance injections of 100 mg each. The total dose received was 397.1 µg over two months. Patients were challenged with subcutaneous PLA₂ followed by a live bee sting one week later. No allergic side-effects were reported and all subjects tolerated the PLA₂ challenge without systemic symptoms. Two peptide-treated patients developed mild systemic allergic symptoms after the bee sting challenge. After therapy, specific proliferative responses by peripheral blood mononuclear cells (PBMCs) to PLA₂ and the peptides were decreased in the three successfully treated patients. The production of Th1 (IL-2, IFN-γ) and Th2 (IL-4, -5, -13) cytokines was also inhibited and B-cells were unaffected in their capacity to produce specific IgE and IgG4 antibodies. Levels increased after allergen challenge in favour of IgG4, as expected with conventional SIT with bee venom. Treatment was associated with the production of IL-10 by peripheral blood mononuclear cells *in vitro*.

MHC-based peptide vaccines

In studies in our own group, a combination of three peptides from the cat allergen Fel d 1, when administered intradermally to cat-allergic asthmatics was shown to induce MHC-restricted isolated late asthmatic reactions (LAR) that appeared to be followed by a period of profound, antigen-specific immunological tolerance [27]. In later studies, 12 overlapping peptides (16 to 17 residues long each) spanning the majority of chains 1 and 2 of Fel d 1, were synthesised. By spanning the majority of the molecule we hoped to increase the number of HLA haplotypes able to bind and present peptide [28]. Using this preparation of peptides it was demonstrated that the magnitude, as well as the frequency, of isolated LARs in cat-allergic asthmatic subjects was dose-dependent. A second injection of peptides was associated with a marked reduction, or absence, of the LAR with a return to baseline values over up to 40 weeks.

More recently, an open study employing an up-dosing protocol with peptides (0.1, 1.0, 5, 10, 25 μg) injected at bi-weekly intervals demonstrated the ability to induce T cell hyporesponsiveness in the absence of LAR's. These results indicate that incremental dosing protocols, starting at a dose that is too low to induce LAR's, may allow peptide immunotherapy to be used safely, without undesirable adverse effects [29].

Following a single peptide injection, cutaneous late phase reactions (LPR) to intra-dermal challenge with whole cat dander extract were significantly reduced [28]. To confirm these observations in a blinded study, 24 cat-allergic subjects were recruited into a double-blind, placebo-controlled study with 16 subjects randomized to peptides via an incremental dosing protocol, to a total dose of 90 µg [30]. Following peptide treatment a significant reduction in both the early and late cutaneous reactions to Fel d 1 and also to whole cat dander were observed, indicating peptide-induced modulation of both B-

cell and/or mast cell respectively. Changes completing treatment and reactions were only appearances were demonstrated cat dander, although cats.

In vitro T-cell response was significantly decreased in peptide-pulsed PBMCs, resulting in decreased production of IL-4. IL-4 deficiency is not only associated with decreased production of Th2 cytokines by PBMCs, but also with decreased cell hyporesponsiveness in a large population of regulatory T cells [10].

Conclusions

An increasing number of studies have shown that peptide immunotherapy of allergic disease through the use of short peptides in combination with adjuvants. The effects is likely to be enhanced by the use of minimal efficacy with the use of multiple epitopes that have broad reactivity and dose interval schedules. The use of hypo-responsiveness to allergens is a broadly applicable in the treatment of cells including autoimmunity and for allergic conditions. The use of peptide therapy from patient and physician therapy.

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cell and/or mast cell (early phase reaction) and T-cell function (late phase reaction), respectively. Changes in late cutaneous reactions were observed within weeks of completing treatment and persisted for several months. Changes in early cutaneous reactions were only apparent at long-term follow-up (3-9 months). No significant differences were demonstrated in bronchial responsiveness to either methacholine or whole cat dander, although peptide-treated subjects were better able to tolerate exposure to cats.

In vitro T-cell responses before and after peptide treatment demonstrated a significant decrease in peptide- and whole allergen-induced proliferation of PBMCs and the production of IL-4, IL-13 and IFN- γ in cultures. Furthermore, peptide-treatment was not only associated with decreases in whole cat dander-induced pro-inflammatory cytokines by PBMCs but also with increased IL-10 production. Thus, induction of T-cell hyporesponsiveness in man may be associated with the induction/expansion of a population of regulatory T cells.

Conclusions

An increasing number of studies in both murine and human models suggest that peptide immunotherapy may offer a safer and effective approach for the management of allergic disease through the modulation of immune responses to allergens. The use of short peptides in order to eliminate/reduce the likelihood of IgE-mediated side effects is likely to be a critical factor determining the success of the approach. For maximal efficacy with the minimum number of peptides, the identification of T-cell epitopes that have broad MHC-binding ability may be important in vaccine design. Dose and dose interval selection may also prove critical for the development of optimal hyporesponsiveness in the absence of adverse events. Peptide immunotherapy may be broadly applicable in all areas in which immunopathology is controlled by T lymphocytes including autoimmune diseases. The ability to deliver safe and effective vaccines for allergic conditions that do not require extensive time and resource commitments from patient and physician may substantially expand the market for disease-modifying therapy.

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Discussion

Malling: It really seems that peptides are not completely efficacious than the original allergen. It may be a number of questions.

Lehrer: Did you use a random digest? If so, it would be a number of questions.

Larché: The peptides are a very good selection? The answer is yes, and as far as we have selected peptides that are effective, twelve peptides were selected from the entire molecule with a high degree of the peptides that are effective, that is why we had to select from cat and other allergens. The selected, purely on the basis of the peptides with Fel d 1 suggest that it is not a question of acting as B cell epitopes in a soluble monomer, but it will not cross-link.

Falagiani: I remember that the derived peptides by the cat allergen, were observed.

Larché: We have no clinical studies, but just in the dose-dependent. So, the chospasm, and monotherapy, drop, and it stays down. agonists. But there is a question.

Falagiani: So, it is a question.

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Discussion

Malling: It really seems to me that the clinical efficacy and the safety of these shorter peptides are not comparable with the old peptides. They have less side-effects and less efficacy than the original peptides. This looks very interesting and there will probably be a number of questions.

Lehrer: Did you use a certain strategy to develop these peptides or was it just a type of random digest? If so, do you know if there were any effects on IgE-binding sites?

Larché: The peptides that we have used were always synthetically produced. Was there any selection? The answer is yes and no. We have always tried to select T cell epitopes and as far as we have understood where those T cell epitopes are located, we have selected peptides that correspond to them. But to some extent the studies with the twelve peptides were done with a sort of shotgun mixture. It was designed to cover the entire molecule with as few peptides as possible, for cost and also logistic reasons. Some of the peptides that were designed for that mixture were insoluble for example, and that is why we had to exclude some of them. But in the future, the peptides derived from cat and other allergens that we are going to evaluate will all be very carefully selected, purely on the basis of them being important T cell epitopes. I think earlier studies with Fel d 1 suggest that some of the peptides that we have in the mixture are capable of acting as B cell epitopes which are dotted all around. I think with this approach it is not a question of excluding B cell epitopes. The question is: Can you deliver peptides in a soluble monomeric form so that they may bind to IgE monovalently and therefore will not cross-link?

Falagiani: I remember that in a clinical study conducted some years ago with Amb a 1-derived peptides by the group of Philip Norman some unusual side-effects, like thoracic pain, were observed. Did you observe something like that in your study?

Larché: We have never observed thoracic pain. We did not do any of those immunologic studies, but just recorded FEV1 after peptide. Peptide-induced bronchospasm is dose-dependent. So if you gave an asthmatic a dose, which is capable of inducing bronchospasm, and monitored the FEV1 during two or three hours, their FEV1 starts to drop, and it stays down for a few hours. This is immediately reversible with beta-2-antagonists. But there is no early-phase reaction from histamine release, for example.

Falagiani: So, it is a dose-related phenomenon?