



Peptide-based immunotherapy: a novel strategy for allergic disease

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KEYWORDS:
allergy, asthma, cytokines, immunotherapy, peptides, T-cells, tolerance

The T-cell component of the antigen-specific immune response is the target of various novel interventions to modify chronic immunologic disorders, such as allergic diseases. Recent clinical trials have evaluated the safety and efficacy of therapeutic vaccines consisting of short, synthetic, allergen-derived peptides, corresponding to T-cell epitopes from the eliciting antigen. The main advantage of such an approach is the reduction in systemic, immunoglobulin E-mediated adverse events compared with existing whole allergen immunotherapy, often referred to as 'allergy shots'. T-cell peptide epitopes, although capable of inducing immunologic tolerance, are short linear structures that have reduced ability to cross-link mast cell- and basophil-bound immunoglobulin E. The precise mechanism of tolerance induction remains incompletely defined. However, recent data indicate that peptide therapy induces/expands a population of antigen-specific regulatory T-cells. A novel form of treatment combining efficacy with a substantially decreased occurrence of adverse events is likely to have a major impact on the management and prevalence of allergic diseases. Furthermore, the principles of epitope-specific therapy hold promise for the development of therapeutic vaccines for the treatment of autoimmune diseases.

Expert Rev. Vaccines 4(6), 881–889 (2005)

The prevalence of atopic allergic sensitization and disease in developed countries has continued to rise since the middle of the 20th century. At present, allergic sensitization rates have reached 40% of the population of some industrialized nations and continue to climb. Asthma, allergic rhinitis, atopic dermatitis and acute expression of allergic disease, such as anaphylaxis, are responsible for a substantial health service burden, particularly in primary care. Allergic diseases are important causes of morbidity and reduced quality of life. Currently available treatment measures include allergen avoidance, pharmacotherapy and 'specific' or whole allergen immunotherapy (SIT). In practice, total avoidance of perennial aeroallergens is difficult, and thus ineffective. Pharmacotherapy is frequently the first form of treatment for mild disease. The drugs most often used as a first step act on end-organ effects, for example, antihistamines, β_2 -agonists and anticholinergics. Glucocorticosteroids are frequently used

as prophylactic agents owing to their ability to downregulate cytokine and chemokine synthesis, with inhibition of cell recruitment and activation. However, patients with severe symptoms, requiring prolonged courses and higher doses, run the accompanying risk of developing systemic side effects.

An alternative for patients who are resistant to conventional pharmacotherapy is SIT, which remains the mainstay of treatment for intractable rhinitis and also insect venom allergy. Treatment involves the administration of increasing doses of allergen extracts, usually via the subcutaneous route. The course is lengthy, taking approximately 3 years, with the ultimate goal being the production of both immunologic and clinical tolerance. SIT is beneficial for rhinitis [1–4], insect venom allergy [5,6] and allergic asthma [7]. Careful selection of patients is essential, as SIT can be associated with a significant risk of immunoglobulin (Ig)E-mediated adverse events,

including systemic anaphylaxis [8]. Furthermore, objective comparisons of clinical efficacy data are hampered by a lack of biologic standardization of allergen extracts.

A desirable form of therapy would be one in which suppression or downregulation of T-helper type 2 (Th2) cell function is achieved without interaction with IgE. A variety of approaches have been explored, including chemical modification of allergens (allergoids), selective usage of allergen isoforms with diminished IgE-binding capacity, recombinantly engineered allergens that have a decreased ability to bind IgE and plasmid DNA vaccines. A further approach targeting antigen-specific CD4⁺ T-cells is immunotherapy using small synthetic peptides representing immunodominant allergen-derived T-cell epitopes. Numerous murine models of peptide therapy have been developed in allergic and autoimmune disease. More recently, translation of this form of therapy into clinical studies has provided evidence of clinical efficacy, and a model in which to investigate mechanisms of immunologic tolerance.

T-cell subsets: role in allergy

The purpose of immunotherapy is the inhibition of inflammatory responses. The role of the T-cell as a key effector in allergic inflammation is well established. T-helper responses to antigens have been divided into two groups based upon the pattern of cytokines produced following activation. Cells secreting cytokines, such as interleukin (IL)-2 and interferon (IFN)- γ , are described as T-helper type 1 (Th1) cells. Th1 responses play a major role in the host response to infection by viruses and bacteria and are frequently associated with autoimmune disease. Cells producing IL-4, 5, 9 and 13 are referred to as Th2 cells. These cells provide help for isotype switching to IgE and are the predominant T-helper phenotype associated with allergic inflammation [9]. Th1 and 2 responses are mutually antagonistic by virtue of their cytokine production profiles. Consequently, inhibition or modulation of allergen-specific Th2 cells and their cytokines has led to the design of novel therapeutic intervention strategies in allergy. A further population of regulatory or suppressor T-cells has recently been identified that secrete cytokines, such as IL-10 and transforming growth factor (TGF)- β , and whose role is currently a major focus of investigation [10,11].

Peptide-mediated immunomodulation *in vitro*

Experiments with human T-cell clones have demonstrated differential activation or hyporesponsiveness, depending on ligand dose. The ability of peptides to modulate the effector functions of T-cells was initially observed for human Th0-type T-cell clones specific for influenza virus hemagglutinin. Exposure of T-cells to supraoptimal concentrations of specific peptide in the absence of antigen-presenting cells (APCs) induced a state of unresponsiveness (anergy) after antigenic rechallenge, which could be reversed by the addition of IL-2 [12,13]. This observation was expanded to include Th2-like cells specific for the house dust mite allergen *Dermatophagoides pteronyssinus* (Der p 1). Downregulation of IL-2 and 4 and maintenance of IFN- γ secretion was additionally observed in these studies [14,15].

Peptide-mediated immunomodulation: murine models

Peptide-induced, antigen-specific nonresponsiveness, or hyporesponsiveness, has been demonstrated in numerous murine models of both autoimmunity and allergy. Initial experiments demonstrated that it was possible to induce systemic tolerance to peptides administered in incomplete Freund's adjuvant (IFA) during the neonatal period [16,17]. A number of subsequent studies have focused on prevention and treatment of experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis (MS). High-dose intravenous peptide delivery resulted in clonal elimination and prevention of disease [18]. EAE was prevented by intraperitoneal administration of immunodominant peptides from myelin basic protein (MBP). Furthermore, treatment with peptides was able to block the progression of disease and decrease disease severity [19]. Metzler and Wraith demonstrated that intranasal, but not oral, administration of MBP acetylated N-terminus (Ac1–9) of the MBP molecule led to protection from EAE [20]. More recently, MBP peptides have been administered repeatedly, intranasally in saline, leading to protection from disease, despite a high precursor frequency of peptide-specific cells in a T-cell receptor (TcR) transgenic model. In this model, protection was dependent upon deletion of effector T-cells and the presence of IL-10 [21,22].

Peptide treatment has also been evaluated in murine models of experimental arthritis. Ku and colleagues administered type II collagen peptide (amino acids 58–73) to both neonatal and adult rats, protecting them from collagen-induced arthritis (CIA) [23]. Staines and colleagues administered 50 μ g of an immunodominant T-cell epitope of type II collagen (amino acids 184–198) intranasally for 4 days prior to the induction of CIA. Peptide treatment delayed disease onset, reduced severity and was associated with induction of IgG₁ antibodies, rather than IgG_{2b} [24]. Prakken and colleagues delivered an immunodominant peptide (176–190) from heat-shock protein (hsp)60 to rats either intranasally (3 x 100 μ g) or subcutaneous (2 x 300 μ g). Rats were subsequently immunized with heat-killed *Mycobacterium tuberculosis* or challenged with the lipoidal amine Avridine. In control rats, both *M. tuberculosis* and avridine led to the development of adjuvant arthritis (AA), whereas hsp60 peptide-treated rats were protected [25]. Chu and Londei treated DBA/1 mice prophylactically with peptides from type II collagen. Peptides were administered intranasally (100 μ g on 3 days consecutively prior to collagen immunization). Mice were either completely protected from disease, or displayed a delayed and less severe disease course [26]. Bayrak and Mitchison demonstrated both prophylactic and therapeutic benefits of multiple intranasal administrations (10 μ g per dose) of the mouse type II collagen peptide p707–721, although the authors expressed reservations concerning the degree and variability of efficacy in this study [27].

Peptide therapy has been evaluated in murine models of type I diabetes. Nonobese diabetic (NOD) mice were protected from the development of diabetes by either subcutaneous peptide (residues 9–23 of the insulin B chain) administration (100 μ g) in (IFA) or intranasal peptide (40 μ g on 3 days consecutively at

4 weeks of age and again at 9 weeks) in saline [28]. Bockova and colleagues demonstrated the ability of two hsp60 peptides (designated p12 and p277) to prevent development of diabetes in NOD mice following subcutaneous administration in IFA [29]. Intranasal administration of glutamate decarboxylase (GAD) peptides before spontaneous development of diabetes in NOD mice resulted in reduced GAD-specific IFN- γ responses and increased IL-5 responses. Mice were protected from disease and CD4⁺ cells from treated mice were able to block adoptive transfer of disease to NOD severe combined immunodeficient (*scid/scid*) mice [30].

Karachunski and colleagues protected mice from experimental myasthenia gravis by intranasal application of peptides from the experimental antigen *Torpedo* acetylcholine receptor (TACHR). Peptides were administered in 50 μ g doses both before immunization with TACHR and during the disease elicitation stage of the model. Peptide-treated mice mounted a predominant Th2 response compared with the Th1 response to antigen observed in control mice. Furthermore, peptide treatment was associated with the induction of anergy in the antigen-specific population in the spleen [31]. More recently, Paas-Rozner and colleagues demonstrated the induction of CD4⁺CD25⁺ regulatory T-cells, with increased expression of cytotoxic T-lymphocyte-associated protein (CTLA)-4 and decreased expression of CD28 following treatment with a peptide consisting of two tandemly arranged analogs of immunodominant epitopes of the human acetylcholine receptor. The analogs represented altered peptide ligands (APL) each containing a single amino acid substitution of the native sequence. Administration of the dual APL reversed disease manifestations in SJL mice [32].

In a rat model of Guillain-Barré syndrome, experimental autoimmune neuritis (EAN), Zou and colleagues demonstrated dose-dependent suppression of disease associated with reduced T-cell proliferation, reduced IFN- γ production and increased TGF- β production. Rats receiving 300 μ g of intranasal peptide (peripheral nervous system myelin component protein P2, amino acids 57–81) per day for 10 days consecutively after disease elicitation were most effectively protected from disease relapse induced by administration of bovine myelin in complete Freund's adjuvant [33].

A number of models of peptide therapy in allergic disease have been developed. Briner and colleagues primed mice with the cat allergen Fel d 1 and demonstrated the ability of allergen-derived peptides to inhibit T-cell cytokine production and antibody responses [34]. Hoyne and colleagues treated mice by intranasal administration of Der p 2 peptides, resulting in the down-regulation of T-cell and antibody responses to the whole protein [35]. Bauer administered a dominant T-cell epitope of Bet v 1 (the major birch pollen allergen) to CBA/J mice both before and after antigen priming [36]. T-cell proliferative responses were inhibited, but there was no change in antibody isotype. In a model of bee venom allergy, mice were sensitized to the major allergen phospholipase (PL)A₂ and treated by intraperitoneal or intranasal administration of peptides covering the

entire molecule. Following intraperitoneal administration, mice were protected from anaphylaxis. Reductions in the Th2:Th1 ratio and allergen-specific IgE were recorded. Administration of peptide before sensitization with allergen resulted in a failure to develop IgE to subsequent immunization with the whole molecule [37].

Generally, experimental models of tolerance have focused on the use of relatively high doses of protein or peptide (greater than 100 μ g cumulative dose) in models of autoimmunity and allergy. Recently, however, tolerance to the male HY antigen was achieved following intranasal administration of only 9 μ g of peptide [38]. In another study, low-dose exposure to influenza HA peptide in a TcR transgenic mouse model resulted in antigen-specific suppression mediated by CD4⁺CD25⁺ regulatory T-cells [39].

Peptide immunotherapy in humans

Cat allergy

A number of groups have undertaken clinical trials evaluating peptide immunotherapy. Norman and colleagues used Fel d 1 peptides to treat cat allergy. Treatment consisted of the subcutaneous injection of an equimolar mixture of two 27 amino acid sequences, IPC-1 and 2 (Allervax®CAT), which contained multiple Fel d 1 T-cell epitopes. A double-blind, placebo-controlled trial involving 95 cat-sensitive individuals was performed, in which four injections of peptides or placebo were administered [40]. Clinical end points of nasal and lung symptoms were measured using a cat room model of allergen challenge, with Fel d 1 levels above 50 ng/m³. Clinical benefit was observed 6 weeks after the end of treatment in the high-dose group. Adverse events, including chest tightness, nasal congestion, flushing and pruritus, were noted a few minutes to several hours after peptide administration. The later side effects were found to diminish in the high-dose group with repeat administration. This finding was consistent with initial T-cell activation followed by induction of T-cell unresponsiveness, and followed precedents from animal studies. Early adverse reactions may have been due to an IgE-mediated mechanism, as the peptides may have retained sufficient tertiary structure to cross-link adjacent IgE molecules on the surface of mast cells.

Further observations from these studies suggested that the peptides may have caused a shift in T-cell functional phenotype, with a decrease in IL-4 production by peptide-specific T-cells taken from subjects in the high-dose group [41]. A separate study using IPC-1/IPC-2 confirmed this finding [42] and additionally observed an improvement in the dose of inhaled allergen required to produce a 20% reduction in forced expiratory volume in 1 sec (FEV₁) (provocative dose; PD₂₀) in the two highest dose groups. However, neither effect achieved statistical significance when compared with placebo. Simons and colleagues, using the same preparation, found no significant differences from placebo in immediate- and late-phase skin sensitivity to whole cat extract [43]. Furthermore, in a multicenter, randomized, double-blind, placebo-controlled study of 133 subjects investigating safety

and efficacy, Maguire and colleagues reported several adverse events, predominantly characterized by respiratory symptoms; three reactions required epinephrine. Again, the magnitude of the events decayed with successive doses. The study failed to demonstrate a significant improvement in end points, with the exception of FEV₁ in subjects with reduced baseline measurements of this index at the start of treatment [44].

Further studies with peptides from Fel d1 used shorter peptides to further reduce IgE interaction. Haselden and colleagues selected three overlapping peptides from chain 1 of Fel d 1; *in vitro* assays with basophils confirmed the lack of histamine-releasing activity of the peptides. Peptides were administered to cat-allergic asthmatic subjects by intraperitoneal injection into the forearm [45]. No local reactions were observed at the site of peptide injection. However, spirometric measurements revealed the induction of isolated late asthmatic reactions (LARs) (defined as a reduction in FEV₁ of $\geq 20\%$) in approximately 25% of subjects. The reactions were attenuated on peptide rechallenge, consistent with the findings of Norman and others using larger peptides. The latter suggested that peptide administration may lead to an initial phase of T-cell activation followed by hyporesponsiveness, or 'tolerance', through mechanisms that may include clonal exhaustion, clonal deletion, anergy or active regulation.

Major histocompatibility complex-restriction of peptide recognition

Using allergen-specific T-cell lines generated from subjects developing isolated LARs, Haselden and colleagues demonstrated major histocompatibility complex (MHC)-restriction of TcR recognition of individual FC1P peptides. Thus, one of the three peptides could be bound and presented to T-cells by human leukocyte antigen (HLA)-DRB1*04, and another by both DRB1*0101 and DRB1*1301/1302. It became apparent that T-cell reactivity depended on HLA molecules restricting peptide presentation, with subsequent induction of isolated LARs in subjects in whom a threshold dose was achieved. The results implied that direct T-cell activation, in a MHC-restricted fashion and in the absence of mast cell/basophil degranulation, could lead to airway narrowing.

Induction of antigen-specific hyporesponsiveness

To study the phenomenon of peptide-induced isolated LARs in a larger proportion of the population, 12 overlapping peptides encompassing the majority of chains 1 and 2 of Fel d 1 and capable of binding to a larger number of HLA molecules were prepared. A preliminary study demonstrated a clear dose-response effect, with an increase in both the frequency and magnitude of reactions observed with an increase in dose [46]. A second administration of peptides was characterized by partial or complete abrogation of the LAR. The late reaction was maximally attenuated between 2 and 8 weeks, with the effect waning over the next 3–6 months. More recently, an open study utilizing an incremental dosing

protocol with the same mixture of peptides injected at biweekly intervals demonstrated the ability to induce T-cell hyporesponsiveness in the absence of LARs, supporting the notion that incremental dosing protocols may allow peptide immunotherapy to be used safely without undesirable adverse effects [47].

The observed reduction in the magnitude of the LAR upon peptide rechallenge prompted investigation of the effects of peptide administration on systemic sensitivity to allergen. A single injection containing 5 µg of each of 12 peptides from Fel d 1 resulted in a significant reduction in the magnitude of the cutaneous late-phase reaction (LPR) following intraperitoneal challenge with whole cat allergen extract [46]. In a subsequent placebo-controlled trial, the same preparation of peptides was administered in incremental doses, with a cumulative dose of 90 µg administered over 2 weeks [48]. Peptide treatment led to a marked decrease in the skin early-phase reaction (EPR) to challenge with purified Fel d 1, and also with whole cat dander allergen extract at a long-term point (3–9 months). The magnitude of the skin LPR was reduced at both short-term (4–6 weeks) and long-term follow-up. Treated subjects were also significantly better able to tolerate exposure to cats after treatment.

T-cell responses to antigen *in vitro* were measured before and after peptide treatment. A significant reduction in peptide- and whole allergen-induced proliferation of peripheral blood mononuclear cells (PBMCs) was observed, together with reduced Th2 (IL-4 and 13) and Th1 (IFN- γ) cytokine production. A concomitant and prolonged increase in IL-10 production was observed [48]. The reduction in proinflammatory cytokines and increased IL-10 suggested that induction of immune regulation, rather than clonal deletion or anergy, may have accounted for the clinical effect of peptide therapy.

Further evidence implicating regulatory T-cells in peptide immunotherapy was observed recently in a study investigating the mechanism of inhibition of the cutaneous LPR to allergen challenge [47]. In skin biopsies, there was a significant increase in the number of double positive CD4⁺CD25⁺ cells following peptide treatment (CD25⁺ cells being activated or regulatory cells). This change correlated with the magnitude of the change in the LPR, suggesting that CD4⁺CD25⁺ regulatory T-cells may mediate peptide-dependent downregulation of the cutaneous response to allergen. Furthermore, a significant increase in IFN- γ -producing CD4⁺ T-cells was also observed, suggesting that at sites of allergen challenge, both recruitment of regulatory T-cells and immune deviation (Th2 to Th1) may be occurring. Interestingly, no increase in IL-10 was observed at skin challenge sites, but there was an increase in expression of TGF- β . It may be, therefore, that the effects of peptide immunotherapy differ depending upon the anatomic compartment investigated. An associated finding in this study was a reduction in airway hyperresponsiveness (measured by histamine PC₂₀), indicating that peptide immunotherapy may be efficacious in the treatment of allergic asthma.

Bee venom allergy

Peptide immunotherapy in bee venom allergy is a particularly attractive concept, as allergic side effects can occur in up to 40% of patients undergoing SIT [49]. Müller administered three short T-cell epitopes of PLA₂, the major bee venom allergen, to five patients who had previously experienced systemic allergic reactions to bee stings [50]. A parallel group of patients receiving SIT acted as control subjects. Following peptide therapy, all subjects were able to tolerate a 10 µg PLA₂ challenge. A wild bee sting challenge under controlled conditions failed to induce a reaction in three of the subjects, whilst the other two had mild systemic reactions. Proliferative responses by PBMCs to PLA₂ and the treatment peptides were decreased in the three successfully treated patients. The production of both Th1 and Th-2 cytokines was also inhibited. Subsequent experiments have demonstrated an increase in allergen-specific IL-10 production in these individuals.

More recently, Fellrath and colleagues treated bee venom immunotherapy using three long synthetic peptides (LSP) encompassing the entire length of the major allergen PLA₂ [51]. A semi-RUSH protocol was employed in which patients received approximately 250 µg in incremental divided doses at 30-min intervals starting with 0.1 µg. Thereafter, maintenance injections of 100 µg were administered at days 4, 7, 14, 42 and 70. In the case of two patients, the maintenance dose was 300 µg, but this was only given until day 42. During the course of peptide therapy, a transient increase in T-cell proliferation to the LSP was observed in the active treatment group only. This was accompanied by increased secretion of IFN-γ and IL-10, but not Th2 cytokines. Allergen-specific IgG₄ levels also increased gradually throughout the study period, in contrast to IgE. Some subjects developed peptide-specific IgE during the course of therapy, and there was no significant change in skin sensitivity to intradermal allergen challenge. Treatment with peptides was generally well tolerated, but in two subjects, local (and occasionally disseminated) erythema was observed, with occasional hand palm pruritis.

Mechanism of action of peptide immunotherapy

Peptide immunotherapy is associated with the induction of 'immunologic tolerance'. However, the precise mechanisms by which this is achieved may vary between protocols and remains unclear in many models. Downregulation of antigen-specific T-cell responses may arise as a result of numerous mechanisms, including clonal deletion, anergy, immune deviation or the action of regulatory T-cells. Induction of clonal deletion may occur after exhaustive activation of effector cells (e.g., after superantigen exposure [52] or antiviral responses [53]), which then undergo apoptotic cell death. A state of anergy may be induced, in which cells are rendered unresponsive to cognate antigen following exposure to the latter in an inappropriate context. For example, T-cells may encounter peptide-MHC complexes in the absence of functional costimulation [54], or presentation of peptide by T-cells or other nonprofessional APCs [55,56]. Redirection of antigen-specific T-cell responses

during specific allergen immunotherapy has been referred to as 'immune deviation' in which, for example, a Th2 response may be suppressed by the action of a simultaneous Th1 response [57]. Finally, active suppression or 'regulation' has been demonstrated in some murine models of peptide immunotherapy [21,38], and more recently in human studies [58]. In this form of immunologic tolerance, proinflammatory responses may be actively downregulated by cell-cell contact between effector cells (or APCs) and regulatory T-cells, or the action of anti-inflammatory cytokines, such as IL-10 and TGF-β. Verhoef and colleagues demonstrated that CD4⁺ T-cells isolated following peptide immunotherapy were able to suppress the antigen-driven proliferative response of CD4^{neg} autologous target cells isolated before therapy [58]. The data suggest that the CD4⁺ population harbors an antigen-specific population of regulatory/suppressor T-cells that were induced by the peptide treatment, supporting the concept that antigen-specific adaptive regulatory T-cells can be induced and expanded *in vivo* [59]. At present, it has not been possible to define the fate of antigen-specific T-cells in this clinical model, and it may be that a combination of clonal deletion, anergy and active suppression contribute to the tolerant phenotype after therapy. Future studies with reagents capable of identifying peptide-specific cells should clarify this issue.

Clinical studies of peptide therapy in both allergic and autoimmune disease have displayed some common mechanistic features. For example, the induction of IL-10 has been reported following peptide therapy in Type I diabetes [60], allergic asthma [48], bee venom allergy [61,62] and rheumatoid arthritis [63]. Reduction of parameters associated with pro-inflammatory responses have also been reported, such as the reduction of proliferative responses of PBMC to antigen [48,62,63] and the reduction of disease-associated cytokines [41,42,48,60,63]. Prakken and colleagues demonstrated increased expression of the regulatory T-cell-associated transcription factor Fox p3 after therapy with hsp peptides in rheumatoid arthritis [25]. A nonsignificant increase in Fox p3 was also reported by Tarzi and colleagues, together with a significant induction of suppressor of cytokine signaling (SOCS)-3, a molecule that suppresses IL-12 production and is induced by IL-10 [62].

Interestingly, the route of administration and dose of peptide may contribute significantly to mechanism of action. For example, in clinical studies in cat allergy, peptides were administered by intradermal injection in the forearm. In this scenario, it is likely that peptides entered the peripheral circulation via the cutaneous capillary bed and trafficked to the lung, where effector T-cells were activated transiently and tolerance followed [46]. In related studies, peptides were administered by inhalation (and demonstrated to induce isolated LAR described previously [64]), but did not lead to tolerance. The presentation of the peptides to the immune system at low dose, high solubility and in the absence of innate immune triggers (e.g., activators of Toll-like receptors [TLRs]) on APCs may render the peptides 'tolerogenic'. Thus, peptides may bind to MHC class II peptide-binding grooves on

the surface of APCs without providing activation/maturation signals [65]. As a result, when immature APCs circulate through lymphoid tissue, they may present the peptides to T-cells whilst in a state of incomplete maturation and activation [66]. By contrast, peptides delivered in small amounts by inhalation or by ingestion may be degraded rapidly, and may not reach the lymphatics or peripheral circulation in sufficiently high concentrations to induce hyporesponsiveness.

Peptide immunotherapy: future approaches

Adaptations of the original model using T-cell peptide epitopes are currently being tested. Many of these newer preparations have yet to reach the stage of clinical trials, but illustrate the wide variety of possibilities available using a peptide approach.

Recombinant Bet v1 fragments & multimers

In a multicenter, randomized, placebo-controlled, double-blind study, Niederberger and colleagues evaluated two genetically engineered forms of the major birch pollen allergen Bet v 1 [67]. An equimolar mixture of two Bet v1 polypeptides (F1 amino acids 1–73; F2 amino acids 74–159) or a trimer of the Bet v 1 molecule were adsorbed to aluminium hydroxide for subcutaneous administration. A total of 127 patients were recruited, although detailed immunologic outcomes were only reported from the largest cohort of subjects treated in Vienna, Austria (n = 66 completed subjects). Bet v 1-specific IgG was significantly increased following treatment with both the polypeptide fragments and the trimer. IgG subclass distribution indicated that IgG₁, IgG₂ and IgG₄ were induced in contrast to conventional allergen extract immunotherapy, after which IgG₄ is the predominant isotype. Evidence was also found of the induction of IgG cross-reactive with Bet v 1-related food and a reduction in symptom scores of subjects with oral allergy syndrome. In basophil histamine release experiments, serum taken from subjects was able to inhibit allergen-induced histamine release. Furthermore, improvement of clinical symptoms and skin sensitivity to allergen correlated with the increase in IgG₁ antibodies observed.

Hybrid peptides

As an alternative to using a mixture of T-cell peptide epitopes, Hirahara and colleagues designed a hybrid peptide [68]. This engineered polypeptide incorporated seven T-cell determinants of Cry j 1 and 2, the major Japanese cedar pollen allergens, linked together in random order to make one polypeptide. Initial *in vitro* studies demonstrated that the hybrid peptide elicited T-cell proliferation in much lower doses than a mixture of the seven peptides. Serum IgE specific for Cry j 1 or 2 was not found to bind to the hybrid peptide, suggesting decreased risk of IgE-mediated side effects compared with native allergen. Results from clinical studies involving this compound have yet to be reported.

B-cell peptide epitopes

In an Austrian study, five synthetic peptides derived from the major B-cell epitopes of Phl p 1, a major timothy grass pollen allergen, were evaluated [69]. The peptides were 28–32 residues

long and demonstrated no secondary or tertiary structure by magnetic resonance scanning. Furthermore, the peptides failed to bind specific IgE antibodies from grass pollen-sensitive subjects, lacked basophil histamine-releasing activity *in vitro* and failed to elicit EPRs in skin prick tests performed on patients. When used in mice and rabbits, the peptides induced protective IgG antibodies, which recognized the whole allergen and group 1 allergens from other grass species. In addition, peptide-induced antibodies inhibited the binding of grass pollen allergic patients' IgE antibodies to the wild-type allergen. Data from clinical studies are awaited.

Th1-skewing peptide analogs

Janssen and colleagues developed a peptide analog of a dominant wild-type allergen epitope and evaluated its therapeutic effect in a murine model of allergic sensitization [70]. Single alanine substitutions produced a Th1-skewing peptide analog that was found to modulate responses of allergen-specific Th2 effector cells, leading to reduced airway eosinophilia and antigen-specific IL-4 and 5 production. Treatment with such analogs may redirect effector Th2 responses toward Th0/1 in allergic subjects.

Conclusions

Currently available therapy for allergic diseases includes symptomatic treatments, such as antihistamines, leukotriene receptor antagonists and glucocorticosteroids, and disease-modifying treatment in the form of allergen immunotherapy. The former approach remains palliative and unable to address the initiation, progression and prevalence of disease. The latter is hampered by adverse IgE-mediated reactions, restricting usage to a minority of patients. Short synthetic peptides representing T-cell (and in some cases B-cell) epitopes may be able to modulate immune responses to allergen, without the risk of frequent adverse events. Peptides of the appropriate size and solubility, administered at the appropriate doses, have demonstrated clinical efficacy in recent studies. Thus, peptide immunotherapy may improve the safety and, perhaps, efficacy of antigen-specific therapy in allergic diseases.

Expert commentary

Allergic diseases are driven by immune responses to well characterized molecules. It follows that the antigen-specific immune response to these molecules offers a uniquely specific target for disease-modifying intervention. Modulation of the immune response to individual proteins has been achieved with specific immunotherapy, but this approach is hampered by allergic (predominantly IgE-mediated) reactions during treatment. The ability to identify T-cell and B-cell epitopes has provided the opportunity to develop synthetic peptide-based vaccines with substantially reduced allergenicity, allowing safer and more rapid treatment. Peptide immunotherapy has been demonstrated to be clinically efficacious in early studies, and larger, Phase III trials are now required. Mechanistic studies have provided evidence for downregulation of

antigen-specific Th1 and 2 responses with concomitant increases in regulatory cytokines, such as IL-10 and TGF- β . CD4⁺ T-cells with regulatory activity have recently been described following therapy. Thus, peptide immunotherapy may be safe and clinically effective in the treatment of both allergic and autoimmune disease.

Five-year view

Clinical studies of peptide immunotherapy have been initiated in several diseases over the past 5 years. Evidence for safety and clinical efficacy is accumulating, and mechanistic studies appear to be pointing in the same general direction; that is, that peptide therapy, regardless of route of administration, leads to the induction of antigen-specific immune regulation through the induction of regulatory T-cells. The ability to induce regulatory T-cells and manipulate antigen-specific responses is critical to

developing disease-modifying therapy in chronic diseases. Over the next 5 years, translation of murine models of peptide therapy into the clinic is likely to expand in both allergic and autoimmune diseases. Current studies employ a variety of dose ranges, routes of administration and dose intervals. Within 5 years, the basic principles of optimal dose and route of administration will be established. Phase III studies are likely to be performed in rheumatoid arthritis, Type I diabetes, asthma and rhinitis (multiple allergen indications). Phase I and II studies should proceed in celiac disease, MS and autoimmune hemolytic anemia. Identification of epitopes driving immunopathology will increase opportunities for clinical intervention. Finally, from a mechanistic point of view, ongoing studies will provide material (e.g., blood) from which regulatory T-cell populations can be isolated and analyzed for phenotype and gene profiling.

Key issues

- Proinflammatory T-cell responses to antigen drive chronic diseases, including allergies and autoimmunity.
- Disease results from a failure to balance proinflammatory (effector T-cell) and anti-inflammatory (regulatory T-cell) responses.
- Peptide administration can result in transient activation of effector T-cells followed by long-lasting tolerance in the antigen-specific T-cell compartment.
- Peptide therapy has been developed in numerous murine models of allergic and autoimmune disease. Tolerance has generally been established though the administration of high doses of peptide (<100 μ g).
- Tolerance takes several days to fully develop after peptide administration.
- Tolerance probably requires a threshold system dose to be achieved.
- Modulation of immune responses to antigen manifest themselves differently in different tissues.
- The induction of antigen-specific regulatory T-cells appears to be responsible, at least in part, for the induction and maintenance of immunologic tolerance.

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