

REVIEW ARTICLE

Immunotherapy with peptides

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Abstract

Specific allergen immunotherapy is clinically effective and disease modifying. It has a duration of effect that exceeds the treatment period and prevents both the progression of allergic rhinitis to asthma and the acquisition of new allergic sensitizations. However, immunotherapy is associated with a high frequency of adverse events related to the allergenicity of vaccines. Allergenicity is conferred by the presence of intact B-cell epitopes that crosslink allergen-specific IgE on effector cells. The use of linear peptide sequences representing fragments of the native allergen is one approach to reduce allergenicity. Preclinical models of peptide immunotherapy have demonstrated efficacy in both autoimmunity and allergy. Translation of this technology into the clinic has gained momentum in recent years based on encouraging results from early clinical trials. To date, efforts have focused on two major allergens, but vaccines to a broader range of molecules are currently in clinical development. Mechanistically, peptide immunotherapy appears to work through the induction of adaptive, allergen-specific regulatory T cells that secrete the immunoregulatory cytokine IL-10. There is also evidence that peptide immunotherapy targeting allergen-specific T cells can indirectly modulate allergen-specific B-cell responses. Peptide immunotherapy may provide a safe and efficacious alternative to conventional subcutaneous and/or sublingual approaches using native allergen preparations.

Specific allergen immunotherapy is clinically effective and has benefit that extends beyond the treatment period (1). Unlike conventional pharmacotherapy, specific allergen immunotherapy is disease modifying and has been shown to prevent the development of asthma (2) and to reduce sensitizations to other allergens (3). Optimal treatment times vary based on allergen and therapeutic preparation, but in general terms, a course of 3 years therapy is regarded as optimal. Although there are no formal published data on the relationship between treatment time and duration of clinical response, it is widely held that clinical benefit will last for twice as long as the treatment period. Thus, treatment for 1 year results in additional benefit for the subsequent year, whereas treatment for 3 years will provide clinical benefit for a further 3 years. The lengthy duration of treatment, the frequent administration of allergen and the associated high frequency of allergic adverse events, which may be systemic and in some cases life-threatening, have limited uptake of this form of therapy. These shortcomings are the result of the allergenicity of the preparations employed for therapy. These contain whole allergen molecules with intact B-cell epitopes that are readily able to crosslink specific IgE molecules on the surface of effector cells (Fig. 1). A number of strategies aimed at reduc-

ing the allergenicity of treatment preparations, whilst maintaining immunogenicity, have been described. Physical modification of allergen molecules may reduce or eliminate IgE reactivity and thus allergenicity (4). Such approaches have taken many forms including chemical modification, conjugation with synthetic bacterial DNA motifs, point mutations in native allergen gene sequences and the use of allergen multimers, fragments and peptides of various lengths. The use of soluble, short synthetic peptides for the treatment of allergic disease allows the delivery of T-cell epitopes of the allergen in an adjuvant-free, tolerogenic form, whilst avoiding IgE-mediated allergic reactions (5, 6). Synthetic peptides have been developed and evaluated in both experimental animal models and human clinical studies. Synthetic peptides are defined active pharmaceutical ingredients (API) and can be produced in a reproducible and standardized fashion that cannot be achieved with allergen extracts. Further advantages include low production costs, ease of purification and good stability in lyophilized form without the need for cold storage.

Presentation of T-cell epitopes to T cells by nonprofessional antigen-presenting cells (APC) results in the induction of 'anergy', a term describing antigen-specific nonresponsive-

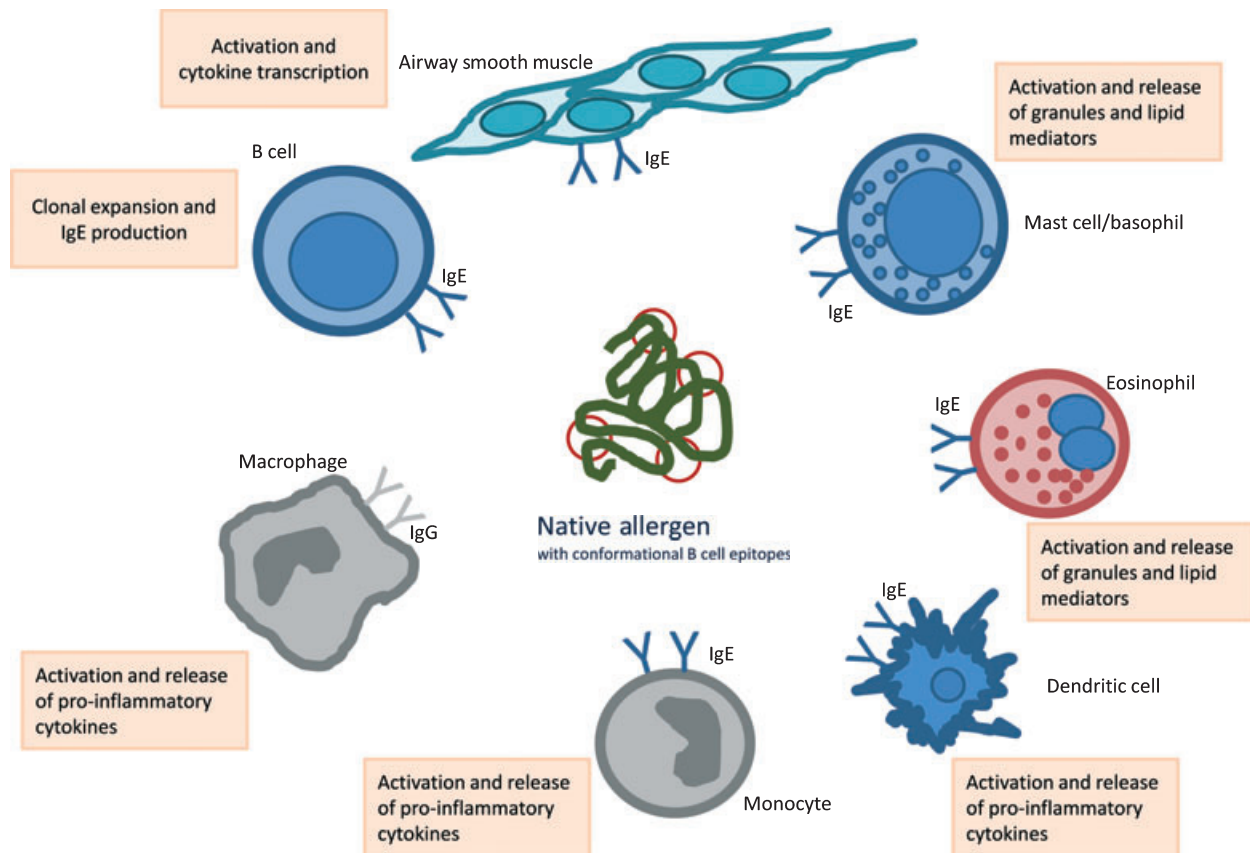


Figure 1 Encounter with native allergen activates effector cells, antigen-presenting cells and structural cells. Cells bearing Fc receptors for IgE and IgG bind allergen-specific antibodies. Crosslinking of cell-bound allergen-specific antibodies by different epitopes (conformational or linear) of the native allergen molecule results in cellular

activation. Mast cells, basophils, B cells, dendritic cells, monocytes and airway smooth muscle cells all express IgE receptors, and macrophages express IgG receptors; all are thus capable of activation during allergen encounter.

ness. For example, when highly purified CD4⁺ T-cell clones were cultured with supraoptimal concentrations of specific epitope, they were refractory to subsequent stimulation with an optimal antigen challenge (7). Similarly, when T-cell clones were cultured with epithelial cells, keratinocytes or myoblasts, expressing MHC class II and specific peptide, anergy ensued (8–10). Indeed, presentation of antigenic peptide by epithelial cells resulted in the generation of T cells with regulatory/immunosuppressive function (10).

Experimental *in vivo* studies of peptide-induced tolerance have been reported in a number of disease areas including allergy, autoimmunity and transplantation. Initially, tolerance was induced in models of autoimmune disease using high-dose, prophylactic administration of peptide in adult or neonatal mice (11–21). More recently, intranasal peptide delivery led to protection from disease, which required deletion of effector T cells and the presence of IL-10, in a murine model of multiple sclerosis (22, 23). Treatment of established disease has also been achieved in allergen sensitization models. Mice were immunized with the house dust mite allergen Der p 2 and tolerized with immunodominant peptides resulting in the down-regulation of both T-cell and antibody responses to

Der p 2 (24). Similarly, mice sensitized to the birch allergen Bet v 1 were rendered tolerant by the administration of peptide-containing T-cell epitopes (25). Mice sensitized to insect venoms have also been treated both prophylactically and therapeutically with peptides to reduce allergic responses following allergen challenge (26, 27). Mice sensitized to cats through priming with the Fel d 1 allergen were treated with two polypeptides derived from the sequence of Fel d 1 chain 1. As a strong adjuvant was used during priming, only Th1 outcomes were assessed. Treatment resulted in the decreased production of IL-2 and allergen-specific IgG (28). More recently, mice lacking endogenous MHC class II but expressing a transgene encoding the human MHC molecule HLA-DRB1*0101 were sensitized with recombinant Fel d 1 and treated with a Fel d 1 peptide known to bind to HLA-DRB1*0101. A single, low-dose (1 µg) of peptide was able to modulate multiple parameters of allergic sensitization including significant reduction in nonspecific airway hyperresponsiveness (AHR), Th2 recruitment and cytokine/chemokine secretion, IgE production, mucus hypersecretion and airway eosinophilia. Tolerance induced by one T-cell epitope was found to confer tolerance to other T-cell epitopes of the same

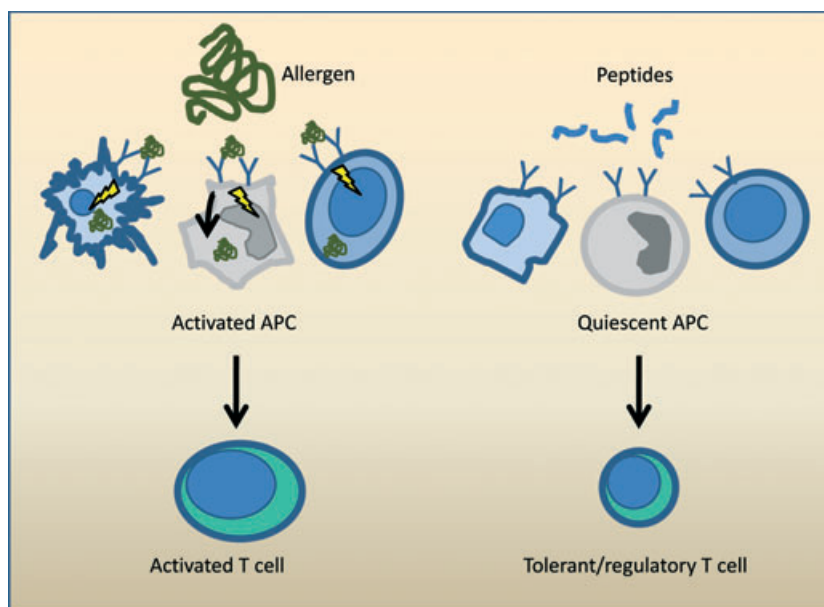


Figure 2 Native allergen, but not short-peptide epitopes, results in the activation of antigen-presenting cells (APC) and inflammatory T-cell responses. Crosslinking of allergen-specific antibodies bound to the surface of APC results in APC activation and upregulation of pro-inflammatory genes. The activated APC presents processed allergen epitopes in an inflammatory context to allergen-specific

T cells. In contrast, short synthetic peptides are unable to crosslink allergen-specific antibodies, resulting in a quiescent encounter between peptide epitope and APC. Presentation of allergen epitopes to T cells in this context results in the induction of immune tolerance.

molecule, providing evidence of the induction of linked epitope suppression. Blocking studies with a monoclonal antibody directed against the IL-10 receptor demonstrated that the treatment effect in this model was IL-10 dependent (29).

Translation of peptide immunotherapy into the clinical setting has been slow but the approach is gaining popularity in the treatment of both allergic diseases and autoimmune diseases. Clinical development programmes are currently underway in multiple sclerosis (<http://www.apitope.com>), type 1 diabetes (30), rheumatoid arthritis (31, 32) and coeliac disease (<http://www.nexpep.com>). However, these programmes are beyond the scope of the current review. Treatment of allergic disease with peptides has, until recently, been limited to cat allergy, ragweed allergy (although these studies have only been published in abstract form) and bee venom. Clinical development programmes are currently underway with other allergens (<http://www.circassia.co.uk>).

Peptides from Fel d 1

Following preclinical studies described earlier (28), a series of clinical trials were performed to evaluate the safety and efficacy of two polypeptides from the major cat allergen Fel d 1. In the first of these to be published, an equimolar mixture of the peptides identified as IPC-1/IPC-2 (AllervaxCat[®]; ImmuLogic Pharmaceutical Corporation, Waltham, MA; equimolar combination of two 27-amino acid synthetic peptides) was compared with placebo in a treatment regimen involving four subcutaneous injections over 2 weeks. Cat-

allergic subjects (with documented allergic rhinitis plus or minus asthma) were treated in 10-fold dose increments (7.5, 75 and 750 µg per injection) (33). Significant improvements in lung and nasal symptom scores were observed in the high-dose group. A related mechanistic study demonstrated reduced IL-4 production in peptide-specific T-cell lines following treatment given by Marcotte et al. (34). A large placebo effect was observed in common with many allergen immunotherapy trials. Despite the observed clinical efficacy, the vaccine was poorly tolerated in many subjects with treatment-emergent adverse events occurring acutely and up to several hours after peptide injection. Most frequently, adverse events were reported in the respiratory system and in particular, subjects with a history of asthma reported symptoms of chest tightness and shortness of breath several hours after dosing. Subsequent investigation identified MHC-restricted activation of allergen-specific effector T cells in the airways as being responsible for these manifestations, highlighting IgE-independent mechanisms for airway narrowing (35). In an inhaled allergen challenge study, small groups of cat-allergic asthmatic individuals were treated with variable doses of IPC-1/IPC-2. The provocative dose of inhaled cat allergen resulting in a 20% reduction (PD₂₀) in forced expiratory volume in one-second (FEV₁) was compared before and after dosing. A significantly higher dose of allergen was required to achieve a 20% reduction in FEV₁ after treatment in the higher dose groups. However, these differences were observed only when comparing pretreatment and posttreatment challenges in vaccine-treated individuals, but not when

these changes were compared with those in the placebo group (36).

Modest clinical improvements were reported in a multicentre study in which cat-allergic subjects received eight subcutaneous injections of high-dose vaccine (750 µg). There was a significant improvement in pulmonary function 3 weeks after therapy, but only in individuals with reduced baseline FEV₁ (37). In common with earlier studies, frequent, acute and delayed adverse events were reported during treatment. Delayed symptoms of asthma (isolated late asthmatic reactions) were reduced with successive doses of peptide suggesting the induction of immunological tolerance. A fourth study in which cat-allergic subjects received four weekly injections of an intermediate dose of vaccine (250 µg) failed to show any significant change in surrogate markers of efficacy or changes in mechanistic outcomes. In common with other studies, treatment was associated with delayed symptoms of rhinitis, asthma and pruritis (38).

More recently, a series of clinical studies have been performed using mixtures of shorter peptides from Fel d 1 (39–44). In a small pilot study, cat-allergic asthmatic subjects received a single intradermal injection of a mixture of 12 short synthetic peptides comprising the majority of the T-cell epitopes of Fel d 1 (45). Skin late-phase reactions (LPSR) to challenge with whole cat dander allergen extract were significantly reduced following peptide treatment. Modulation of skin responses was associated with reductions in allergen-specific proliferation and a reduction in both Th1 and Th2 cytokines (39). In a double-blind, placebo-controlled clinical trial, an identical mixture of Fel d 1 peptides was given in a multiple dose, incremental fashion (cumulative dose = 90 µg) to a small cohort (n = 24; 16 active, 8 placebo) of cat-allergic asthmatic subjects. In common with the earlier study, statistically significant reductions were observed in the LPSR to whole allergen challenge. Additionally, at later follow-up (3–9 months posttherapy), the magnitude of the early-phase skin response (EPSR) was also significantly reduced. Significant within-group changes in quality of life observed that these changes did not achieve statistical significance when compared with placebo. Changes in clinical surrogate markers were associated with reduced allergen-specific responses of peripheral blood mononuclear cells (PBMC) *in vitro*, including reduced proliferation and reduced secretion of both Th1 and Th2 cytokines. A concomitant and significant increase was observed in PBMC secretion of the regulatory cytokine IL-10. No acute, treatment-related adverse events were reported in this study, but isolated late asthmatic reactions were expected (based on the dose of peptides employed) and observed in some subjects. Induction of isolated late asthmatic reactions did not appear to be related to the induction of allergen-specific tolerance (40). Related mechanistic studies using PBMC isolated from subjects treated in the trial demonstrated that proliferative and cytokine responses to sequences within Fel d 1 that did not form part of a peptide vaccine were also significantly reduced. These findings are indicative of intramolecular tolerance (also known as ‘linked epitope suppression’) and imply that peptide vaccines need

not necessarily contain all T-cell epitopes from a target antigen to achieve broad antigen-specific tolerance (29).

Changes in nonspecific AHR were addressed in two studies, with differing outcomes. In a small, open-label study using a similar peptide preparation delivered at 2-week intervals and using a lower dosing regimen, a significant reduction in airway hyperreactivity (measured by PC₂₀) was observed (41). In contrast, no change in AHR was observed in a slightly larger, double-blind, placebo-controlled study (40). The complex nature of AHR and the marked variability of longitudinal measurements within patients make this outcome difficult to assess in small, underpowered clinical studies.

Most recently, a seven peptide vaccine (Toleromune Cat®; Circassia Ltd., Oxford, UK) has been evaluated in a phase IIa safety and tolerability study (46). Peptide components were evaluated in direct MHC binding assays (10 commonly expressed MHC alleles) to determine which sequences would provide the best population coverage. Peptides displaying promiscuous binding to MHC class II were assessed in proliferation, cytokine (IFNγ, IL-10, IL-13) and histamine release assays, and vaccine components were selected. The resulting vaccine was administered, without adjuvant, to 88 cat-allergic individuals either intradermally or subcutaneously to evaluate safety and tolerability and, as a secondary outcome, the dose of peptide resulting in the greatest inhibition of the LPSR to whole allergen challenge as a surrogate of clinical efficacy. The vaccine was safe and well tolerated and, importantly, had an adverse events profile that was indistinguishable from placebo providing confidence that future clinical trials will be adequately blinded in contrast to current approaches. Cohorts of eight cat-allergic subjects received a single injection of vaccine (n = 6) or placebo (n = 2). Intradermal delivery gave a maximal (40%) reduction in the LPSR at a dose of 3 nmol of vaccine, similar to earlier findings with the prototype vaccine (39, 46). Phase IIb clinical studies are now underway to further evaluate the safety and tolerability of a vaccine and to begin to explore clinical efficacy.

Peptides from Api m 1

Only three studies of peptide immunotherapy in bee venom-allergic individuals have been reported. None of these were fully blinded, placebo-controlled studies, and each study was performed in small numbers of individuals. However, the data that emerge strongly support the findings of earlier clinical interventions with peptides from Fel d 1. Immunodominant T-cell epitopes of the major bee venom allergen Api m 1 were identified (47) and subsequently used in a pilot clinical study to treat five bee venom-allergic subjects who had documented (moderately severe) systemic allergic reactions to bee venom. Subjects received incremental doses (cumulative dose 397.1 µg) of an equimolar mixture of peptides at weekly intervals (48). Ten subjects treated with standard bee venom immunotherapy acted as a control group. All five subjects tolerated a subcutaneous challenge with 10 µg of whole Api m 1 following the last treatment injection. Subsequently, a wild bee sting challenge was performed, which was tolerated

by three of five subjects, with the remaining two developing mild systemic allergic reactions.

Texier et al. (49) used direct peptide-MHC binding assays to quantify the binding affinities of overlapping synthetic peptides spanning the Api m 1 sequence. Four immunodominant peptides were identified, three of which were similar to those used previously for therapy by Müller and colleagues. Tarzi et al. (50) used these peptides (formulated in saline) to perform an open-label, single-blind study of peptide therapy in subjects with mild bee venom allergy (subjects had not experienced systemic reactions following bee sting). The peptides were well tolerated, and no treatment-emergent adverse events were observed. In common with studies performed using Fel d 1 peptides, treatment resulted in reduced proliferative and cytokine (Th1 and Th2) responses in PBMC cultured with allergen. In contrast, IL-10 secretion was significantly up-regulated. Furthermore, LPSR to both whole bee venom and Api m 1 were significantly reduced.

Fellrath et al. (51) treated bee venom-allergic subjects with a RUSH desensitization protocol using three long synthetic peptides (LSP) encompassing the entire Api m 1 sequence. Starting at 0.1 µg, subjects were administered approximately 250 µg in incremental doses at 30-minute intervals. Maintenance injections of 100 or 300 µg were administered on days 4, 7, 14, 42 and 70. A transient increase in T-cell proliferation to the treatment peptides was observed, together with increases in IFNγ and IL-10 levels, but not Th2 cytokines. Allergen-specific IgG₄, but not IgE, levels increased throughout the study period, likely due to the presence of some intact B-cell epitopes within the polypeptides. Peptide-specific IgE was induced during treatment in some subjects, reminiscent of seasonal increases in IgE to allergens during the pollen season. In contrast to a number of studies with Fel d 1 peptides, no significant change in skin sensitivity to intradermal whole allergen challenge was observed. Peptide therapy was generally well tolerated, although local and disseminated erythema with hand (palm) pruritis was observed in two subjects.

Evidence for the induction of regulatory T cells during peptide immunotherapy

Reductions in the LPSR to allergen challenge were a consistent feature of several studies employing mixtures of short, synthetic peptides. Immunohistochemical analysis of skin biopsy sections following skin allergen challenge demonstrated a significant increase in CD25⁺ cells and CD4⁺/IFN-γ⁺ cells after peptide therapy, suggesting that the recruitment of Th1 cells (and perhaps regulatory T cells) to sites of allergen encounter may contribute to the mechanism of action of these vaccines. In contrast to PBMC responses following culture with whole allergen, no increases in IL-10⁺ cells were observed in allergen-challenged skin biopsies; however, the expression of TGFβ mRNA was increased suggesting that different mechanisms of tolerance may dominate at different anatomical locations (41).

Evidence of a role for the immune regulatory cytokine IL-10 and modulation of the allergen-specific B-cell response fol-

lowing peptide immunotherapy has also come from studies employing peptides from Api m 1. Increased IL-10 production in cultures of PBMC incubated with allergen was observed in studies employing both short and LSP from Api m 1 (50, 51). Both studies were also associated with an increase in allergen-specific IgG₄, although this was modest with the use of short peptides. Although no measurements of IL-10 were made in the earliest of these studies (48), changes in the isotype of allergen-specific antibodies indicative of an increase in IL-10 were observed. Initially, no change was observed in the levels of allergen-specific serum IgE or IgG₄ during the course of treatment in this study. However, following subcutaneous challenge with Api m 1, a week after treatment, the concentration of both isotypes, particularly IgG₄, increased. These data suggest that whilst therapy with short peptides may not directly stimulate allergen-specific B cells (because of the inability of the peptides to crosslink B-cell surface antigen receptors), encounter of whole allergen molecules following the induction of IL-10-secreting, allergen-specific T cells results in the redirection of B-cell responses to IgG₄ through the action of IL-10, which has previously been shown to potentiate the production of this isotype (52).

The effect of peptide immunotherapy on regulatory T-cell function was evaluated using PBMC obtained at baseline and following the completion of treatment in a double-blind, placebo-controlled trial (43). Treatment was associated with significant reductions in allergen-specific proliferative responses and IL-13 production from PBMC *in vitro*. Measurement of the functional regulatory activity of CD4⁺CD25⁺ cells to suppress allergen-specific proliferative and cytokine responses was assessed by mixing these putative regulatory T cells with autologous CD4⁺CD25⁻ cells. Peptide immunotherapy did not alter the suppressive activity of purified CD4⁺CD25⁺ cells suggesting that naturally occurring regulatory T cells may not play a significant role in the immunological changes associated with this form of immunotherapy. These experiments were performed prior to the identification of more specific markers of regulatory T cells such as the CD4⁺CD25^{hi}CD127^{lo} phenotype and it is thus likely that the putative regulatory population isolated in this study was a mixture of regulatory cells and recently activated T effector cells. However, recent data from murine studies of low-dose peptide immunotherapy support the conclusion that natural (thymus-derived) regulatory T cells may not play a significant role in peptide immunotherapy as no increase was observed in Foxp3⁺ T cells in the lungs of mice after treatment (29).

A role of antigen-specific inducible regulatory T cells in the efficacy of peptide immunotherapy was addressed through the isolation of CD4⁺ T cells (containing the putative regulatory cells) from PBMC samples obtained before and after peptide immunotherapy. CD4⁺ cells were cultured with CD4^{negative} cells that had been labelled with the cell division-tracking dye (44). CD4⁺ T cells from posttreatment samples suppressed the antigen-driven proliferation of CD4^{negative} cells from pretreatment samples, demonstrating that peptide immunotherapy induces a population of CD4⁺ T cells with suppressive/regulatory activity. Assembling

the available data from a limited number of studies, it appears that low-dose peptide immunotherapy may induce IL-10-secreting adaptive regulatory T cells (akin to Tr1 cells) that are capable of down-regulating Th2 responses to allergen. However, in this form of treatment, there is little evidence of a major role for natural CD4⁺CD25⁺Foxp3⁺ regulatory T cells.

Mechanisms of peptide-induced tolerance

The induction of T-cell anergy and T cells with immunosuppressive function following presentation of peptides by non-professional (such as myocytes and keratinocytes) or immature (dendritic cells) APC supports the notion that antigen recognized by T cells under noninflammatory conditions results in tolerance (53). Indeed, tolerance to both exogenous (54) and endogenous antigens (55) may be maintained, at least partly, by pools of regulatory T cells that secrete IL-10. Administration of highly soluble peptides in the absence of pro-inflammatory adjuvants may exploit this in intrinsic tolerance pathway (Fig. 2). Furthermore, administration of peptides lacking sufficient secondary structure to crosslink adjacent immunoglobulin molecules may further reduce the likelihood of creating an inflammatory milieu during T-cell recognition of peptide–MHC complexes.

Conclusions

Peptide immunotherapy represents one strategy to reduce the allergenicity of therapeutic preparations for the treatment of allergies. Short, synthetic peptides containing T-cell epitopes show markedly reduced ability to crosslink allergen-specific IgE. The resulting reduction in allergenicity has allowed more recent clinical trials to be performed in a truly blinded fashion in contrast to studies of subcutaneous and sublingual immunotherapy performed with intact allergen preparations. Peptide immunotherapy modifies numerous surrogate markers

of allergen exposure such as skin responses to allergen challenge, nonspecific AHR (some studies), symptom scores following inhaled allergen challenge, quality of life and the ability to tolerate natural allergen exposure. Peptide immunotherapy appears to induce a population of functional regulatory/suppressive cells with characteristics of Tr1 cells. Peptide immunotherapy, in human subjects, is associated with the induction of IL-10. In experimental murine models, where such experiments are possible, tolerance has been shown to be dependent upon IL-10. Peptide immunotherapy has a variable effect on the B-cell compartment with larger peptides (which presumably retain more B-cell epitopes) being better able to induce allergen-specific IgG₄ responses than short peptides.

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Author contributions

DM and ML conceived and wrote this manuscript. ML produced the figures.

Conflict of interest

The author is a founder, shareholder and consultant of/to Circassia Ltd, a company developing peptide-based immunotherapy. The author is the scientific founder of Adiga Life Sciences Inc., a joint venture between Circassia Ltd and McMaster University.

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