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STATE OF THE ART REVIEWS ON MECHANISMS OF ALLERGIC DISEASE

Immunoregulatory T cell epitope peptides: the new frontier in allergy therapy

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Summary

Allergen immunotherapy (AIT) has been practised since 1911 and remains the only therapy proven to modify the natural history of allergic diseases. Although efficacious in carefully selected individuals, the currently licensed whole allergen extracts retain the risk of IgE-mediated adverse events, including anaphylaxis and occasionally death. This together with the need for prolonged treatment regimens results in poor patient adherence. The central role of the T cell in orchestrating the immune response to allergen informs the choice of T cell targeted therapies for down-regulation of aberrant allergic responses. Carefully mapped short synthetic peptides that contain the dominant T cell epitopes of major allergens and bind to a diverse array of HLA class II alleles, can be delivered intradermally into non-inflamed skin to induce sustained clinical and immunological tolerance. The short peptides from allergenic proteins are unable to cross-link IgE and possess minimal inflammatory potential. Systematic progress has been made from *in vitro* human models of allergen T cell epitope-based peptide anergy in the early 1990s, through proof-of-concept murine allergy models and early human trials with longer peptides, to the current randomized, double-blind, placebo-controlled clinical trials with the potential new class of synthetic short immune-regulatory T cell epitope peptide therapies. Sustained efficacy with few adverse events is being reported for cat, house dust mite and grass pollen allergy after only a short course of treatment. Underlying immunological mechanisms remain to be fully delineated but anergy, deletion, immune deviation and Treg induction all seem contributory to successful outcomes, with changes in IgG_4 apparently less important compared to conventional AIT. T cell epitope peptide therapy is promising a safe and effective new class of specific treatment for allergy, enabling wider application even for more severe allergic diseases.

Introduction

Allergic diseases constitute a global health problem affecting an estimated 20% of the population (up to 40% in some countries). There are many different triggers of allergic diseases and clinical patterns range from mild allergic rhinitis to potentially life-threatening asthma and anaphylaxis. Allergic diseases inflict a huge socio-economic burden, exaggerated by their typically chronic nature. Currently, there is no cure. Available pharmacotherapies, including antihistamines, bronchodilators, corticosteroids and the newer biologicals, aid symptom relief and adrenaline provides emergency treatment of anaphylaxis. To date, the only proven form of disease-modifying treatment is allergen immunotherapy (AIT). The goals of AIT are to induce sustained immunological and clinical tolerance to the allergen following cessation of treatment [1–3]. Current clinical regimens comprise repeated, often incremental, doses of whole allergen extracts via subcutaneous injection (SCIT), or sublingual drops or tablets (SLIT), often over several years.

Efficacy of AIT was first reported by Noon et al. [4] in the early 1900s in studies of grass pollen allergy. Since then, administration of whole allergen extracts for AIT has become accepted clinical practice for treat-

ment of allergy to many aeroallergens and insect venoms (wasps, bees). Different forms and delivery routes of allergen have been trialled, but currently only whole allergen extracts are licensed for clinical practice, with SCIT, where indicated, remaining the most effective route [5, 6].

Despite the success of AIT in appropriate individuals, there remain major concerns with safety, efficacy and adherence [7]. These result from the complexity of allergen extracts, prolonged treatment courses, and the risk of adverse events due to intact allergens with retained IgE reactivity. Several approaches to reduce allergenicity of whole allergen molecules, without affecting immunoregulatory activity, have been explored including allergoids, recombinant allergen derivatives and allergen fragments, some with evidence of clinical efficacy [8–13]. However, of particular interest and the focus of this review is the development of short T cell epitope-based peptides as a potential new class of pharmacotherapy for allergic diseases. Constituent peptides are designed to comprise immunodominant T cell epitopes with negligible IgE-binding and lacking inflammatory cell stimulatory capacity. Their presentation in a non-immunogenic form induces long-lasting allergen-specific T cell non-responsiveness after only a short course of treatment. Here, we retrace the origins of this therapy from the initial seminal reports of *in vitro* high-dose T cell epitope peptide-induced anergy in human allergen-specific T cells in the 1990s to proof-of-concept murine allergy models of anergy and early clinical studies. Finally, recent highly encouraging clinical trials of T cell epitope peptide therapies and associated data on immunological mechanisms are reviewed.

The rationale for T cell targeted therapy for allergic diseases

Refining effective immunological therapies for allergic diseases requires detailed understanding of the underlying immune response to allergens, especially factors that influence whether adverse reactions or tolerance ensues. Allergic reactions are caused by inflammatory mediators released from activated mast cells, basophils and eosinophils, processes driven by allergen cross-linking of cell-bound specific IgE and Th2 cell-derived cytokines: IL-4 and IL-13 switch allergen-stimulated B cells to produce IgE antibodies; IL-5 promotes eosinophil migration and activation in the skin and mucosae; IL-3 and GM-CSF promote eosinophil differentiation and, together with IL-4 and IL-9, the maturation and activation of mast cells and basophils [14–16]. Pathogenic allergen-specific Th2 cells can be further characterized by surface marker phenotype. Wambre et al. showed that $CD27^{-}CRTH2^{+}$ allergen-specific Th2 cells could be identified in grass pollen-allergic subjects, but not healthy controls and that this T cell population was preferentially lost following effective SCIT [17, 18]. In contrast, allergen-specific Th1 and Treg (particularly IL-10 producing Tr1) subsets predominate in non-atopic subjects, or those with resolved clinical symptoms following conventional AIT [16–21]. High levels of IFN-γ and IL-10 are induced at sites of allergen exposure following successful AIT, augmenting Th1-reactivity whilst inhibiting Th2 cell proliferation. IFN-γ and IL-10 also promote B cell production of specific IgG_1 and IgG_4 antibodies that can inhibit formation of allergen-IgE complexes and prevent IgE-facilitated antigen presentation by B cells, further down-regulating adverse Th2-type inflammatory responses [22, 23].

T cell epitope peptide therapy harnesses the fundamental immunological ability of peptides comprising dominant T cell epitopes to induce anergy and/or deletion of specific T cells. Specific anergy relies on the functional cytokine plasticity of Th cells in order to allow down-regulation of pathogenic effector T cell responses as well as inducing naive T cells to mount protective responses [24, 25]. Other properties of allergen-specific T cells contribute to feasibility of this approach for treatment of allergy. Firstly, conserved repertoires of T cell epitopes of allergens were noted within a given individual when screened over 2 years [26] in contrast to changing T cell specificities over time for autoantigens [27]. Secondly, analysis of the human T cell repertoire reveals a bias in both the TCR-Vα and TCR-Vβ gene segment usage, as well as *in vivo* clonal dominance by long-lived house dust mite (HDM)-specific T cell clones [28]. Persistent grass pollen-specific T cell clones have also been demonstrated *in vivo* [29]. Importantly, T cells from the same clonal origin can 'switch' from dominant IL-4 production to dominant IL-10 or IFN-γ production during *in vitro* anergy induction or conventional AIT [29–31]. Taken together, these data strongly suggest that inactivation or elimination of dominant monoclonal populations of pathogenic allergen-reactive T cells would modify beneficially the immune response to allergen and observed clinical phenotype.

T cell epitope mapping of allergens and selection of peptides for a therapeutic

Identifying $CD4^{+}$ T cell epitopes within allergens is mandatory for the design of T cell targeted therapeutics. T cell epitope mapping requires knowledge of the allergen sequence and isolation or identification of allergen-specific T cells from allergic donors, both of which have been greatly facilitated in recent years by the evolution of more sophisticated and/or high-throughput methodologies. Most major allergenic proteins have now been cloned and sequenced [see www.allergen.org, register of validated data maintained by the Allergen Nomenclature

Sub-Committee of the World Health Organization and International Union of Immunological Societies (IUIS)], allowing synthesis of nested sets of peptides spanning the entire allergen sequence to determine sites of T cell reactivity, as described below. Due to low precursor frequencies of allergen-specific T cells in peripheral blood, analysis of T cell epitopes of allergens is facilitated by prior enrichment of allergen-specific T cells. Initially, this was achieved by limiting dilution of allergen-stimulated whole PBMC to obtain clonal T cell populations [32, 33]. New methodologies for analysing T cell responses to allergen peptides include flow cytometry techniques with proliferation dyes such as carboxyfluorescein diacetate succinimidyl ester (CFSE) to detect proliferating cells by their reduced staining intensity [34, 35], cytokine capture kits [36] and fluorochrome-conjugated HLA class II-peptide tetramers [29, 37]. Carboxyfluorescein diacetate succinimidyl ester-based approaches provide a highly sensitive method for detecting T cell responses, particularly when used together with other activation markers, such as CD25, but bystander proliferation can decrease specificity [38] necessitating validation of potential T cell epitopes in large patient cohorts. ELISPOT-based approaches are useful for high-throughput screens of whole PBMC [36] and can also be used for core T cell epitope mapping using T cell lines or clones [39, 40]. T cell epitope mapping using peptide-stimulated PBMC cultures (as opposed to T cell lines and clones) is feasible if the assay is rigorously designed and appropriate statistical methods are used. However, few such studies have been performed on allergens (e.g. [37]).

HLA-peptide tetrameric complexes facilitate the identification and characterization of allergen-specific T cells without the need for expression of particular functional activities, providing a sensitive and specific tool for analysis of peptide-specific T cells directly *ex vivo* [29, 37]. However, generation of tetramers is expensive and currently only a small proportion of HLA class II molecules are available in this form. Importantly, tetramers cannot map precise core T cell epitopes for optimal T cell stimulation to inform selection of the shortest and safest peptide set for therapy (see below). In contrast to CFSE-approaches, tetramer-based approaches show very high specificity, but sometimes lack sensitivity [38]. Similarly, *in silico* algorithms can be used to predict $CD4^+$ T cell epitopes by identifying theoretical HLA class II binding motifs within protein sequences based on analyses of thousands of known epitopes [41]. However, whilst such algorithms can provide preliminary guides cost-effectively, they are not comprehensive and predicted HLA-binding motifs require validation by analysis of peripheral blood T cell responses [38, 42].

To identify all potential T cell epitopes, allergen-specific T cell lines and clones generated from a large patient cohort are screened for reactivity against overlapping synthetic peptides spanning the entire sequence of the allergen molecule, each usually 15–20 amino acids in length with overlaps ranging from five amino acids upwards. Following identification of T cell reactive peptides, precise core epitope sequences are mapped utilizing peptide sets truncated from the N- and C-termini, for example as demonstrated in early studies for a rye grass pollen allergen Lol p 5 T cell epitope [43]. Minimal core $CD4^+$ T cell epitopes are typically eight or nine residues long, but lengths for optimal T cell stimulation may be longer and vary between subjects. This likely reflects varied requirements for flanking residues in stabilizing different HLA-peptide-TCR complexes and increasing persistence of the peptide at the APC surface [44–46]. Peptides selected for immunotherapy tend to range from 12 to 20 residues, consistent with naturally processed peptides eluted from HLA class II molecules [47, 48].

T cell reactive sites have been mapped for many allergens and are catalogued in The Immune Epitope Database (www.iedb.org [49, 50]). A meta-analysis of this database confirmed 1406 allergen-derived $CD4^+$ T cell epitopes based on human T cell reactivity [51]. However, despite the large number, these represent less than 17% of all allergens in the IUIS allergen database (www.allergen.org). T cell epitopes are typically found throughout an allergen sequence, but responder frequency evaluations from large subject cohorts assign dominance [41], underpinning design of T cell targeted peptide therapeutics. Dominant T cell epitopes also typically have the strongest T cell stimulatory capacity, an important consideration for immunotherapy following the established immunological dogma that the strongest immunogens are the strongest tolerogens [52]. As specific allergic immune responsiveness in atopic individuals is not typically limited to a single dominant epitope, careful mapping of the critical minimal set of immunodominant T cell epitope peptides is essential. In addition to frequency of reactivity, peptide selection criteria can include patterns of reactivity, reproducibility of T cell response and, importantly, ability to induce a response in patient PBMC. In some cases, where two epitopes are in close proximity, the inclusion of both in a single peptide is desirable provided the final peptide length is kept below about 20 amino acid residues. For some closely related allergens, for example group 1 grass pollen allergens, cross-reactive T cell epitopes have been identified [53–57] which may be advantageous for obtaining broader acting therapeutics with applicability in different world regions.

For therapeutic production, some peptides require modification to ensure solubility and stability for ease of manufacture and administration. This may include modification of terminal residues and substitution of cysteine residues with alanine or other non-reactive

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residues such as serine to avoid potential peptide aggregation (e.g. [35]). In these cases, T cell reactivity of the modified peptide must be reconfirmed. Importantly, for safety of the therapeutic, all candidate T cell epitope peptides must finally be tested singly and in combination to ensure lack of ability to bind and cross-link inflammatory cell-bound IgE. A convenient and reliable assay to assess clinically relevant, functional IgE reactivity is the basophil activation test by flow cytometry or histamine release [58–61].

HLA class II restriction of T cell recognition of allergen peptides

A further important consideration when selecting candidate peptides for immunotherapy is whether the peptides can be presented to T cells by different HLA class II molecules and hence be suitable for targeting genetically diverse populations. $CD4^+$ T cells recognize a specific epitope only when it is complexed with a particular HLA class II molecule encoded by one of three highly polymorphic loci, HLA-DR, HLA-DP or HLA-DQ. One strategy to inform HLA types likely to bind a known T cell epitope utilizes T cell epitope prediction algorithms. Previously, such algorithms could predict binding only to HLA-DR molecules, but recent advances now endorse HLA-DQ and HLA-DP predictions [41]. However, as with T cell epitope mapping, such predictions require experimental validation using isolated HLA molecules and/or transfected L cells or EBV-transformed B cell lines homozygous for defined HLA alleles [26, 41, 62–65]. Analysis of HLA-peptide binding can indicate clinically relevant specificity as well as avidity and/or affinity of the interaction [41, 65, 66].

Whilst algorithms can predict peptide binding to particular HLA types and some assays confirm binding, it is important to demonstrate the full repertoire of functional HLA-peptide complexes. Evidence of functionality requires assays of T cell proliferation or cytokine production for the given HLA-peptide complex. Initial broad determination of HLA-restriction specificity of T cell epitope recognition can be made using blocking monoclonal antibodies specific for HLA-DR, HLA-DP or HLA-DQ [26, 35, 62]. Tetramers provide another method for screening for T cell reactivity to a given HLA-epitope complex in samples such as blood analysed directly *ex vivo* [37]. Unfortunately, screens utilizing tetramers or homozygous cell lines require HLA-matched $CD4^+$ T cells/patients which can be logistically challenging [67]. Furthermore, many HLA molecules are hard to isolate and use in tetramers, thus limiting the range of HLA types that can be tested.

Unlike some autoimmune conditions, allergic diseases generally are not closely linked with particular HLA types [68]. Reflecting this fact, allergen T cell epitopes often demonstrate extensive HLA-binding degeneracy and, in turn, allergen-specific $CD4^+$ T cells may recognize a particular epitope complexed with several different HLA class II molecules [18, 35, 36, 40, 41, 69–71]. Importantly, whilst nominal antigens are most commonly presented on HLA-DR molecules, many allergen T cell epitopes have been shown to also be presented on HLA-DQ and HLA-DP molecules [26, 28, 35, 40, 41, 59, 64, 72, 73]. This is highly advantageous for a therapeutic as HLA-DQ and HLA-DP subtypes are more conserved across populations than HLA-DR molecules; for example, HLA-DP*0401 and 0402 alleles are together present in ~ 50% of the Caucasian population [74].

Experimental models of allergen T cell epitope peptide immune modulation

Functional inactivation of allergen-reactive human T cells in vitro

O'Hehir et al. [75] first reported T cell epitope peptide induction of anergy in allergen-specific T cells in the 1990s. Incubation of cloned HDM-specific T cells with supraoptimal concentrations of their specific ligand, resulted in decreased proliferation to a subsequent immunogenic challenge, decreased IL-4 and IL-5 synthesis but maintained IFN-γ and IL-10 production [30, 75]. During the induction phase of anergy by allergen T cell epitope peptides, there was transient release of some chemokines and Th2 cytokines (IL-4 in particular) suggesting a period of hyperexcitation before the development of sustained anergy [30, 31]. Cytolysis was not the mechanism in this model as T cells were responsive to exogenous IL-2 [75]. Loss of allergen-dependent proliferation and altered cytokine production was accompanied by down-regulation of TCR, and upregulation of CD2, CD25 and adhesion molecules such as LFA-1 [76, 77]. There was also blunting of the typical upregulation of CD28 observed in activation [78]. Altered signalling pathways underlying defective TCR signalling were demonstrated by abrogated activity of $p56^{lck}$ and ZAP-70 tyrosine kinases in a bee venom allergen (PLA2)-specific $CD4^+$ T cell model [79].

Functional inactivation of allergen-reactive T cells in vivo *using murine models*

Prior to clinical development of the T cell epitope peptide therapies, murine models of allergy were developed to validate the strategy and further explore mechanisms. Using a murine model of HDM allergy, inhalation of the immunodominant T cell epitope peptide of Der p 1 by naïve or sensitized mice inhibited the T cell response not only to the peptide but to

whole Der p 1, termed linked suppression or infectious tolerance [80] (Fig. 1). This is of particular importance when contemplating clinical potential in novel AIT [81]. At the same time, there was a report of peripheral T cell tolerance in naïve and primed mice following subcutaneous injections of T cell epitope peptides from Fel d 1, the major cat allergen [82]. Subsequently, similar findings confirming the robustness of anergy, linked suppression and changes in cytokine functional phenotype induced by dominant T cell epitope peptides were reported for murine models of allergy to birch pollen [83], Japanese cedar pollen [84, 85], olive pollen [86], bee and hornet venom [87], bee venom [88], cat [89], egg-white [90] and Timothy grass pollen [91]. In some studies, anergy and regulatory activity were shown to coexist [80, 92]. Using HLA-DR1 tetramers to track allergen-specific T cells in a murine model of cat allergy, Campbell et al. demonstrated Fel d 1 T cell epitope peptide-induced linked epitope suppression associated with IL-10^{+} T cells [89]. Mackenzie et al. [93] used adoptive transfer of Th2-polarized cells in a murine ovalbumin TCR transgenic model to explore the effects of peptide immunotherapy on antigen-experienced T cells. They showed preferential effects on cytokine production by CD62L^{lo} cells (effector and effector memory T cells) rather than CD62L^{hi}Th2 cells (associated with central memory T cells) in suppression of airway inflammation.

Clinical translation of T cell epitope-based peptide immunotherapy

Clinical trials of allergen-specific peptide immunotherapy have been conducted for allergy to bee venom and several aeroallergens, including recent phase IIb and III trials providing strong proof-of-concept and informing further development of this therapeutic strategy.

Bee venom allergy

In a pilot study of peptide immunotherapy for bee venom allergy, five bee venom-allergic patients were treated by subcutaneous injection with three T cell epitope peptides of the major bee venom allergen PLA2, each of 11–18 amino acid residues [94]. Consistent with linked suppression, clinical efficacy was achieved to a subsequent PLA2 challenge and live whole bee sting challenge. In follow-up studies, a semi-rush regimen of three long synthetic peptides encompassing the entire PLA2 sequence was followed by maintenance doses for up to 70 days [95]. Although increased Th1 cytokines and allergen-specific IgG$_4$ were achieved during the study period, some subjects developed peptide-specific IgE and two subjects developed local erythema with occasional palmar pruritus. These findings emphasize the importance of using the shortest possible peptides comprising T cell epitopes to minimize the risk of IgE-mediated adverse events.

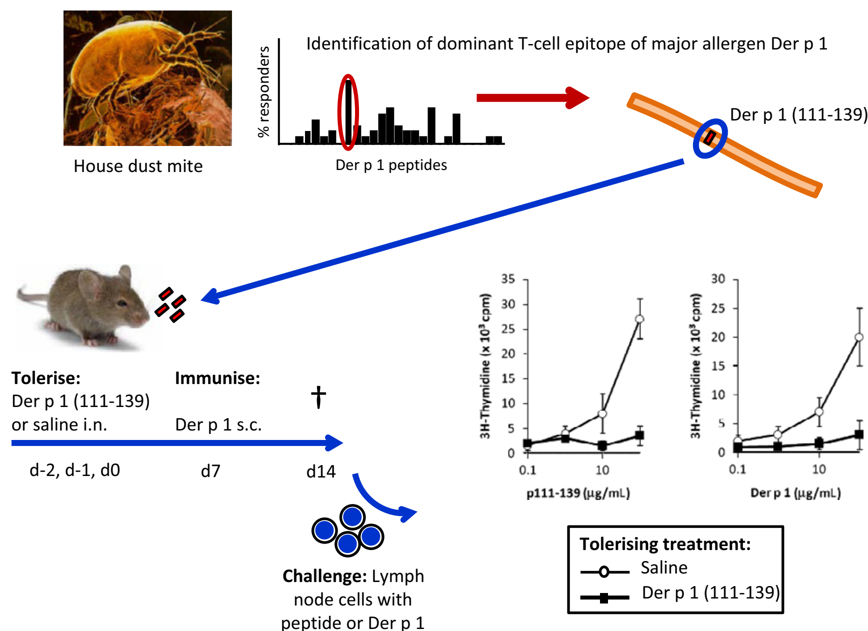

Fig. 1. Linked epitope suppression by T cell peptide therapy in a murine model of allergy. Naïve mice were treated intranasally (i.n.) with the dominant T cell epitope peptide Der p 1(111–139) (tolerizing treatment), or with saline as a control, and then immunized with Der p 1 by subcutaneous (s.c.) injection. Immunogenic challenge of lymph node cells with peptide or Der p 1 in culture showed that inhalation of the dominant T cell peptide had induced T cell anergy/tolerance to the specific ligand as well as the intact house dust mite protein (adapted from [80]). This therapy was also effective in allergen-sensitized mice.

Cat allergy – first generation peptides

Early clinical trials of peptides for cat allergy showed variable efficacy, but large protein determinants were trialled rather than minimal epitopes. The early Fel d 1 peptides comprised an equimolar mixture of two long 27 amino acid sequences from the two chains of Fel d 1 and contained multiple T cell epitopes [96]. A double-blind placebo-controlled trial with 95 cat-allergic subjects was conducted using four subcutaneous injections of the peptide mixture (Allervax®CAT) or placebo [97]. Clinical benefit was demonstrable at 6 weeks but adverse events included nasal congestion, flushing, pruritus and chest tightness for minutes to hours after peptide delivery. The possibility of retained conformational structure within the long peptides and IgE-mediated reactivity likely explained the early adverse events. The asthmatic responses, in subjects with or without underlying asthma, were subsequently attributed to cytokine release from peptide-stimulated T cells [98], consistent with the initial T cell stimulation and cytokine flare observed early in the induction phase of allergen peptide-induced anergy *in vitro* [30, 31], recognizing that IL-4 is a bronchoconstrictor. The delayed adverse effects diminished after repeated delivery. In another clinical trial using the same Fel d 1 peptides, several adverse events, including late asthma responses requiring adrenaline in three cases, were also observed [99]. These early studies with longer peptides given at very high concentrations subcutaneously were disappointing also in failing to achieve evidence of sustained clinical efficacy [99, 100].

Synthetic peptide immuno-regulatory epitope therapy

Newer promising research pioneered by Larche and Kay in the late 1990s and early 2000s led to a second generation of T cell epitope-based peptides for allergy therapy, now designated as synthetic peptide immuno-regulatory epitopes (SPIRE) [98, 101]. These comprise short peptide units, typically 13–17 amino acids in length, administered at lower concentrations (≤ 12 nmol; ~ 75 vs. 750 μg) via the intradermal route into non-inflamed skin [60, 101–105]. The first SPIRE, Cat-PAD (Circassia Ltd; Oxford, UK), comprises seven T cell epitope-based peptides (13–17 amino acids in length) derived from Fel d 1. It is produced as a lyophilizate and reconstituted in water for intradermal administration. A non-injectable device for transdermal delivery has been utilized in the most recent clinical trials which comprise four treatments at 4 week intervals before challenge testing and measurement of total rhino-conjunctivitis symptom score in an environmental exposure chamber (EEC). Early-phase studies demonstrated safety and clinical efficacy [60, 104]. The shortness of the peptides avoids any potential for IgE-cross-linking or inflammatory cell activation and careful dose adjustment seems to avoid the late asthma response observed earlier with the longer peptides. In a recent phase III clinical field study, enduring clinical efficacy was demonstrated out to 2 years after one course of treatment with cat-PAD [106]. As seen with whole extract SCIT and SLIT studies [107, 108], a substantial placebo effect was observed, but this was not sustained over the longer term and efficacy with the cat-PAD therapy was significantly higher. SPIRE therapies are currently being trialled with similar encouraging results from early-phase IIb studies for HDM [109, 110], grass pollen [111] and ragweed pollen [112].

Mechanisms of T cell epitope-based peptide therapies from clinical studies

As clinical translation of T cell peptide therapy for allergy progresses, the underlying immunological mechanisms are being elucidated [113, 114]. Although some mechanisms appear to overlap with conventional AIT, there seem to be distinct differences from current SCIT or SLIT (Fig. 2) [16, 115]. As for AIT with whole allergen, down-regulation of T cell proliferative and cytokine response to allergen is a consistent observation following peptide immunotherapy (e.g. [101, 105]), but the precise mechanism underlying this altered response is not clear. In the early bee venom T cell peptide clinical study, the decreased PLA2-specific T cell proliferation and decreased IL-2, IL-4, IL-5, IL-13 and IFN-γ production were reversed by IL-2 and IL-15, suggesting anergy as the mechanism [94]. However, distinction between re-activation of anergized T cells and activation of naive T cells or indeed Treg could not be made due to the polyclonal nature of the cultures and limited phenotyping. Deletion of allergen-specific T cells is an alternative mechanism suggested from murine models of peptide-induced tolerance. A recent study used HLA class II tetramers to quantify allergen-specific clonal T cell populations *ex vivo* following conventional allergen SCIT for grass pollen allergy [18]. Preferential loss of clonal Th2-type T cells specific for dominant epitopes of major grass pollen allergens over T cells specific for less-dominant epitopes with a Th-1 or Tr1-phenotype was observed. A potential caveat of tetramer-based approaches is their reliance on detection of the TCR on the cell surface with the possible confounder of inability to distinguish between deletion and anergy given that there is down-modulation of TCR expression on anergic T cells. However, in this study, the pathogenic Th2 cells were further distinguished by lack of CD27 expression providing another marker to confirm selective loss of these cells. Furthermore, these cells also had decreased expression of the apoptotic inhibitor Bcl-2

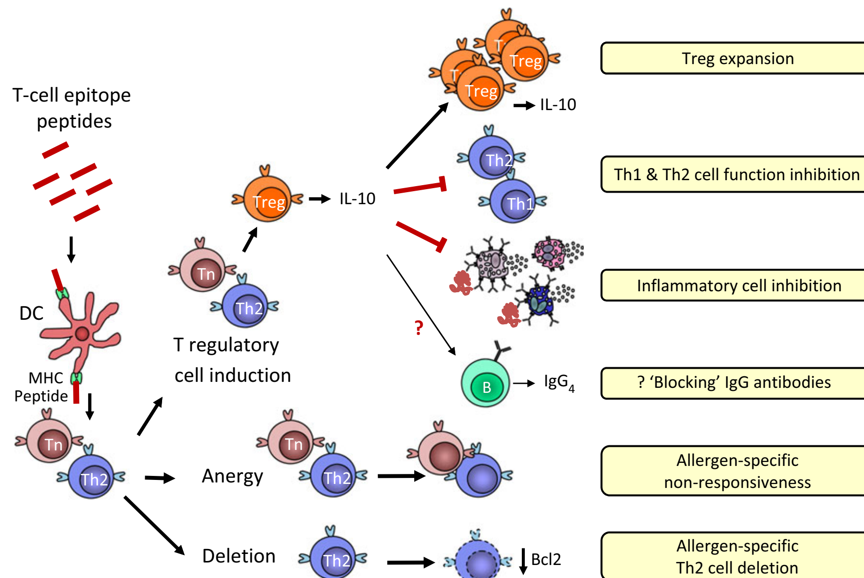

Fig. 2. Immunological mechanisms of allergen T cell epitope-based peptide therapy. Murine and human studies suggest that down-regulation of the adverse Th2-polarized response to allergen by high-dose allergen T cell epitope peptide treatment is mediated by anergy of allergen-specific naïve $CD4^+$ T cells (Tn) and Th2 cells, deletion of allergen-specific Th2 cells and/or induction of Treg with IL-10 production, further expanding Tregs and inhibiting Th1, Th2 and inflammatory cell function. $CD4^+$ T cells show functional cytokine plasticity depending on the conditions of activation and cytokine milieu. The role of IgG_4 blocking antibodies in T cell peptide-mediated clinical tolerance is unclear.

over the cells that escaped deletion. Together these data suggest that dominant T cell epitope-based peptides of major allergens can cause targeted and desirable inactivation or deletion of the most pathogenic T cells in allergic subjects.

As mentioned earlier, the observed late asthmatic responses experienced with the first generation Allervax trials for cat allergy using high concentrations of peptides are likely due to the surge of Th2 cytokine release, specifically IL-4, early in the induction phase of anergy [30, 31]. The lack of asthma on continued administration would be consistent with the lack of continued IL-4 secretion in established anergy. It may be that persistent antigen exposure is required to maintain the anergic state *in vivo*, either naturally in the environment as would be expected for cat, HDM and pollens or by booster antigen encounter.

Increased production of IL-10 and induction of Treg are the most frequently reported mechanisms underlying conventional AIT. Similarly, efficacy of allergen peptide immunotherapy, including early bee venom studies, early Allervax trials and SPIRE therapy, was associated with increased IL-10 production during therapy and a role for Treg is indicated [101, 116]. In clinical studies on cat allergen peptide therapy, IL-10 was shown to be required for peptide-induced suppression of allergen-specific immune responses and linked epitope suppression [89], and induction of an antigen-specific $CD4^+$ T cell population with regulatory function was demonstrated [117]. Analysis of skin from sites of allergen challenge showed an increased number of $CD4^+IFN\text{-}\gamma^+$ and $CD25^+$ cells after peptide therapy suggesting roles for immune deviation and regulatory T cells [118]. It should be noted that interpretation of many studies of Treg subsets and function, especially using clinical samples, may be difficult due to overlap of surface marker expression between Treg and Teff, especially when activated. Further functional analysis and phenotyping of peripheral blood and tissue T cells are required to distinguish activated $CD4^+$ T cells from natural or induced Treg [119].

There is less evidence for induction of specific IgG_4 blocking antibody with successful peptide immunotherapy. Peptides used for AIT, in particular SPIRE, are short and screened for lack of IgE binding and inflammatory cell activating potential, so are unlikely to drive antibody production. However, subsequent exposure to the whole allergen in the context of an altered specific immune response could potentially result in production of specific IgG_4 or IgA antibodies. In the first bee venom study, antibody responses were found to be unaltered during peptide therapy, but further subcutaneous whole allergen challenge caused an increase in specific IgG_4 antibodies [94]. In contrast, in a subsequent study by Tarzi et al., the challenge-induced increase in specific IgG_4 was marginal and transient [116]. Further follow-up studies after peptide therapy are required to assess the importance of allergen-specific IgG_4 antibodies in establishing long-term clinical efficacy. Although blocking antibodies are considered

important in allergen desensitization with whole allergen AIT, their requirement for durable tolerance induction is debated [113, 120], and their desirability in the treatment of allergy to potent allergens such as peanut and shellfish is questioned.

Future prospects for T cell epitope-based peptide therapy

Although the underlying immunological mechanisms that underpin successful allergen T cell epitope-based peptide immunotherapy are still being elucidated, the positive outcomes of clinical trials using EEC coupled with absence of IgE-mediated adverse events augur well for future utility in the clinical setting. Accumulating data suggest a shared role with conventional AIT of Treg induction, IL-10 effects and immune deviation with a less convincing body of data around a role for IgG blocking antibody. The long-lasting clinical efficacy after only four intradermal doses, together with the early side-effect of late asthma with the first generation longer peptides, suggests to us that anergy may be a key mechanism. Co-administration of a beta agonist during the initial dosing to avoid any bronchoconstriction from T cell IL-4 release is worthy of consideration, as this side-effect was seen in both asthmatic and non-asthmatic patients. The point of change in functional phenotype of the anergized T cells following release of the Th2 cytokines is desired, and hence, blockage of any associated transient airway reactivity would be appropriate to allow the desired sustained therapeutic outcome.

Recent trials give confidence that delivery of the therapeutic by an intradermal route to non-inflamed skin is highly efficient in achieving the desired outcomes without the risk of anaphylaxis that frequently and unpredictably accompanies conventional whole allergen extract AIT and the newer forms of whole food extract oral immunotherapy. Final refinement of patient-friendly transdermal delivery devices, optimal concentration and dosing intervals of T cell peptide therapy for specific allergies is awaited with anticipation.

Conclusion

Taken together, the growing body of data from clinical trials in a range of allergic disorders supports the view that a new class of T cell peptide therapy for allergic diseases is imminent. Dominant T cell epitope-based allergen peptides seem to be particularly able to induce sustained immunological and clinical tolerance. Importantly, core epitope mapping informs selection of the critical short amino acid sequences of specific allergens that can provide the desired tolerance without the undesired effects of IgE-cross-linking and inflammatory cell activation. The well-established high level of degeneracy of binding of these allergen peptides to a range of HLA class II molecules further supports the ability to manufacture a population-based therapy, rather than requiring detailed patient endotyping and individualized medicines. Moreover, the demonstration of long-lasting sustained efficacy after only a short treatment course without adverse events raises optimism that the discipline of allergology is on the brink of a new era in allergen therapeutics.

Conflict of interest

The authors declare no conflict of interest.

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