

Concepts and perspectives on peptide-based immunotherapy in allergy

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Key words

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Abstract

Allergen-specific T cells play a key role in the pathogenesis of allergic diseases through provision of help for allergen-specific B cells and control of inflammatory responses. Allergen immunotherapy using intact allergen proteins (given either subcutaneously or sublingually) is clinically effective and demonstrates enduring efficacy (i.e., disease modifying). However, the requirement for monthly injections or daily sublingual administration (both for 3 years), combined with a high frequency of local and systemic adverse events, results in poor compliance. Targeting allergen-specific T cells with synthetic peptides representing dominant T cell epitopes markedly decreases treatment times (4–8 intradermal injections), reduces adverse events and provides efficacy for at least 2 years. We have developed peptide immunotherapies for allergies triggered by cats, house dust mites, and grass pollen.

Each of these consists of a mixture of seven peptides containing multiple dominant T cell epitopes and each have demonstrated statistically significant improvements in rhinoconjunctivitis symptom scores in controlled allergen challenge facilities. The mechanisms of action appear to involve increased IL-10 production, intra- and inter-molecular suppression, and down-regulation of chemokine pathways. In contrast, treatment does not appear to be associated with deletion of allergen-specific T cells, nor with the induction of allergen-specific IgG (as is seen with conventional whole allergen immunotherapy).

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Development of peptide immunotherapy

The first studies describing the use of T cell epitopes for the treatment of allergic diseases date back to the early 1990s. In 1993, two separate studies showed in murine models that the administration of T cell peptides derived from Fel d 1, the major cat allergen, or Der p 1, a major house dust mite allergen, were able to suppress the recall responses to the respective allergens both in naive and sensitized mice [1, 2]. Based in part on these findings, Norman and colleagues described the treatment of 95 cat-allergic subjects in a double-blind, placebo-controlled trial evaluating 4-weekly subcutaneous injections of two 27-amino acid peptides from chain 1 of Fel d 1 (referred to as IPC-1 and IPC-2). Administrations of 750 µg of the two peptides showed clinical efficacy, with improved lung and nasal symp-

toms after exposure to live cats [3]. However, 16 of 24 subjects treated with the 750 µg dose developed adverse respiratory events up to 3 hours after the treatment. While some of these reactions were likely due to cross-linking of allergen-specific IgE by residual tertiary structure in the long peptides, others were most probably isolated late asthmatic reactions (LAR) driven by T cell recognition of high doses of Fel d 1 epitopes in subjects with asthma. The T cell dependence of these reactions was later demonstrated by Haselden and colleagues [4]. Other studies of IPC-1/2 reported variable efficacy but similarly high incidence of adverse events [5, 6, 7]. Related trials in ragweed allergy were reported only in abstract form [8].

In unrelated studies and using substantially shorter peptides (between 11 and 18 amino acids in length),

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Müller and colleagues administered peptides representing immunodominant T cell epitopes from the major bee venom allergen phospholipase A₂ (Api m 1) to five bee venom allergic individuals. Treatment was administered by subcutaneous injection of escalating doses of peptides totalling almost 400 µg of each peptide. After treatment, subjects tolerated intradermal injection challenge with 10 µg of Api m 1. Subsequently, three subjects also tolerated a bee sting challenge without substantial clinical reaction, the other two experiences mild systemic reactions. Analysis of T cell responses after treatment showed diminished allergen-specific proliferative responses and decreased production of Th1 and Th2 cytokines [9]. Some years later, Tarzi and colleagues employed a similar, but expanded, set of peptides to treat 12 bee venom allergic individuals in an open-label controlled study [10]. Therapy reduced surrogate clinical outcome markers such as the magnitude of the cutaneous late-phase skin reaction to intradermal allergen challenge and also reduced peripheral blood mononuclear cells (PBMC) production of Th1 and Th2 cytokines whilst increasing the immunosuppressive cytokine IL-10.

Whilst investigating mechanisms associated with late-onset symptoms of asthma associated with the

IPC-1/IPC-2 studies described above, Haselden and colleagues demonstrated that intradermal delivery of a mixture of short (16–17 amino acid) peptides from the sequence of Fel d 1 to cat allergic asthmatic subjects was able to induce a form of allergen-specific T cell tolerance that waned with time [4]. In a series of follow-up studies using a larger panel of peptides and culminating in a randomized, double-blind, placebo-controlled trial, intradermal treatment with Fel d 1 peptides was shown to reduce early-phase and late-phase cutaneous responses to allergen challenge [11, 12] to improve visual analogue assessment of symptoms [12] and to increase methacholine PC20 [13].

Over time, methods for the identification of dominant T cell epitopes at the population level and the selection of appropriate candidate peptides for treatment have become increasingly sophisticated. Recent strategies have tended to utilize mixtures of short peptides (between 13 and 17 amino acids long) that are not physically able to crosslink IgE, but are still capable of T cell immunomodulation. However, some clinical development programs have evaluated/continue to evaluate larger peptide fragments of allergens with evidence of biological activity but also associated adverse events [14, 15, 16].

In general terms, short peptides identified for therapy within our own group have been selected on the basis of desirable characteristics which include good solubility, little or no ability to release histamine from blood basophils of allergen-sensitized subjects, and the ability to bind with moderate or high affinity to multiple MHC class II gene products, such that the great majority of the allergic population can recognize at least one of the peptides in the mixture. The epitopes to be considered for the mixture are identified initially by *in silico* and *in vitro* MHC-binding assays and the results are validated by *ex vivo* T cell activation assays performed on blood samples of allergic donors [17].

Whilst the intradermal route is generally avoided in whole allergen immunotherapy (subcutaneous immunotherapy, SCIT) due to the density of blood vessels and mast cells that can increase the risk of systemic side effects, the preferred route for the administration of the peptides has been the intradermal one. In non-inflamed skin, the large number of quiescent antigen presenting cells, mostly dendritic cells (Langerhans cells and dermal dendritic cells), present in the dermis provide the best environment for an efficient and tolerogenic presentation of the peptides to the T cells.

Safety and efficacy of peptide immunotherapy

Among the major advantages of peptide immunotherapy, as compared to conventional whole-allergen approaches, is a reduced incidence of adverse

Abbreviations

AIT	Allergen immunotherapy
ALLERVAX CAT	Synthetic cat allergen peptides
Cat-PAD	Cat peptide antigen desensitization
COP	Contiguous overlapping peptides
CPT	Conjunctival provocation test
EEC	Environmental exposure chamber
EEU	Environmental Exposure Unit
EPSR	Early-phase skin responses
GINA	Global Initiative for Asthma
HDM	House dust mite
LAR	Late asthmatic reaction
LPSR	Late-phase skin response
PBMC	Peripheral blood mononuclear cell
PD20FEV1	Provocative dose that induces a 20 % fall in FEV1
PLA2	Phospholipase A2
SCIT	Subcutaneous immunotherapy
SLIT	Sublingual immunotherapy
TRSS	Total rhinoconjunctivitis symptom score

events allowing for a shorter course of treatment since greater molar doses of peptide compared to whole allergen can be administered. As few as 4 injections over 3 months of treatment has proven sufficient to persistently reduce allergic rhinoconjunctivitis symptoms, which compares favorably to conventional whole allergen treatments that require monthly (SCIT) or daily (sublingual immunotherapy, SLIT) administration of allergen for three years or more (**Fig. 1**). To date, peptide immunotherapy for the treatment of cat, house dust mite (HDM), grass, and ragweed allergies has been tested in phase 2b clinical trials with positive results. In all of the clinical trials (**Tab. 1**) of peptide immunotherapy described below, safety and tolerability were good, with the frequency and severity of adverse events in placebo and active treatment groups being indistinguishable.

Cat allergy

Building on earlier studies with prototype formulations, Worm and colleagues described the identification and selection of T cell epitopes from Fel d 1 for the formulation of a seven peptide mixture (Cat-PAD, cat peptide antigen desensitization) for the treatment of cat allergy [17]. In the first-into-man phase 2a placebo-controlled, double-blind clinical trial with 88 cat allergic subjects, two different routes of administration (subcutaneous and intradermal) were evaluated using an escalating dose cohort design. No severe adverse events were reported and the injections were well tolerated up to the highest dose; 12 nmol for the intradermal administration and the 20 nmol dose for the subcutaneous administration. The intradermal route was preferred as it showed a greater reduction of late-phase skin reactions induced by intradermal challenge with

whole allergen extract (surrogate clinical outcome), even after a single administration of peptides.

Subsequently, the clinical efficacy of the treatment was evaluated in two separate phase 2b environmental exposure chamber (EEC) studies. In the first trial, 121 cat-allergic subjects were randomized in 5 groups and received placebo (8 injections), or one of the following 4 regimens of treatment with Cat-PAD: 4 injections $\times$ 3 nmol, 2 weeks apart; 4 injections $\times$ 6 nmol, 2 weeks apart; 4 injections $\times$ 3 nmol, 4 weeks apart; 8 injections $\times$ 3 nmol, 2 weeks apart. Changes in the total rhinoconjunctivitis symptom score (TRSS) between baseline and 18–22 weeks after the start of the treatment were used as primary endpoints. The TRSS was measured over 8 nasal and ocular symptoms, each of which scored between 0 (no symptoms) and 3 (severe symptoms), occurring during environmental exposure to airborne cat allergen extract for 3 hours each day, over 4 consecutive days. The group receiving 8 injections $\times$ 3 nmol, 2 weeks apart, had the greatest decrease in mean change in TRSS compared to baseline (mean difference of 2.9 units from 1 hour of exposure onwards from day 2 to 4). No serious adverse events were reported and other adverse events occurred at the same frequency in placebo and active groups [18].

The second trial followed a similar study design, using an EEC: two hundred and two subjects were randomized in 3 groups, receiving placebo (8 injections) or Cat-PAD; 8 injections $\times$ 3 nmol, 2 weeks apart, or 4 injections $\times$ 6 nmol, 4 weeks apart. The prospectively defined primary efficacy variable for the study was the mean change in TRSS at 50 to 54 weeks from 1–3 hours on days 2 to 4 of in the post-treatment challenge compared with baseline values for non-asthmatic subjects. At this time

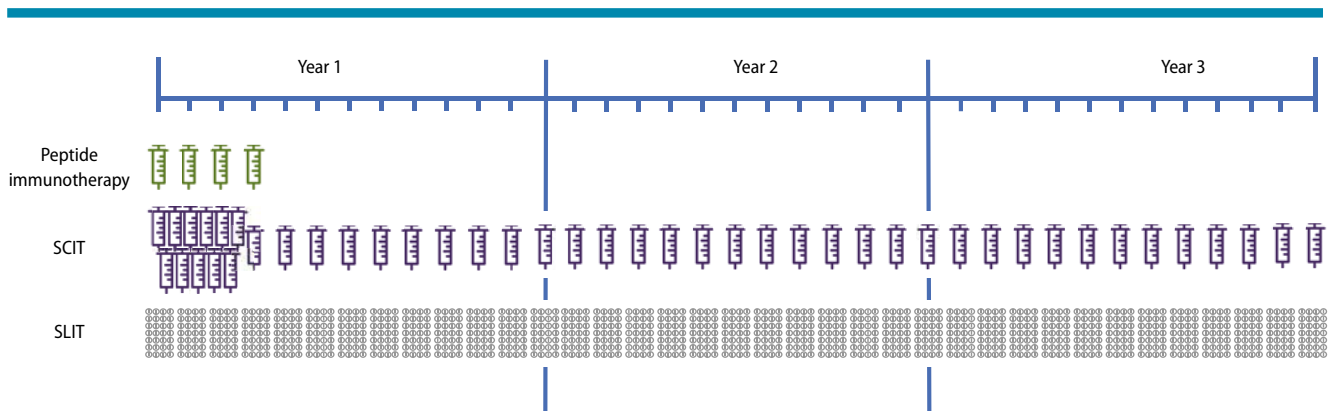


Fig. 1: Treatment course comparison between peptide immunotherapy and conventional whole allergen approaches. Usually, peptide immunotherapy requires as little as four intradermal administrations, four weeks apart. Subcutaneous immunotherapy (SCIT) requires weekly injections of the allergen for the first few months, followed by monthly injection for three or more years. In sublingual immunotherapy (SLIT), the allergen is administered daily, in tablet or droplet form, for three or more years.

point, mean TRSS had decreased in the 4×6 nmol group (-6.778) compared to both placebo (-2.908) and the 8×3 nmol groups (-3.893). The changes in the 6 nmol dosing group were statistically different to those in the 3 nmol group ($p = 0.03$) and the placebo group ($p = 0.01$). Interestingly, at the earlier 18–22 week follow up time point, the two active groups had a similar decrease of the TRSS compared to baseline (8×3 nmol = -5.136 ; 4×6 nmol = -5.406 ; placebo = -2.786) suggesting that both doses initially displayed equivalent efficacy, but that the 3 nmol dose was insufficient to maintain this effect out to 1 year.

Analysis of the total study population (non-asthmatic and asthmatic subjects) revealed similar treatment effects. There was a larger decrease in mean change from baseline TRSS for the 6 nmol group (-6.353) compared to the 3 nmol group (-3.636) and placebo (-2.488). The change in TRSS in the 6 nmol group was statistically significantly different to the 3 nmol group ($p = 0.03$) and the placebo group ($p = 0.006$). Again, in this study the treatment was well tolerated, with the only severe adverse event reported in the placebo group and not related to the drug [19].

Following a protocol amendment, 51 subjects from the same clinical trial were followed in a blinded fashion for a further year at which time they were re-challenged in the same EEC under identical conditions between 102 and 106 weeks (~ 2 years) from the beginning of the study. No further treatment had been received beyond the initial 3 month treatment period. The results showed a persistent effect of the treatment even after 2 years, with the improvement in mean TRSS from baseline in the group receiving the 6 nmol dose still evident compared to placebo (-5.87 versus -2.02) [20].

HDM allergy

Peptide immunotherapy for HDM-induced allergic rhinoconjunctivitis was evaluated in a first-in-man phase 2a study in HDM-allergic subjects: The treatment was safe and well tolerated and all 4 active treatment arms (4 administrations, 4 weeks apart of 0.03, 0.3, 1.0, 3.0, and 12 nmol of peptide mixture) resulted in a reduction of the conjunctival provocation test (CPT) response after challenge (between -16.7% to -41.4% , no change in the placebo group). The largest change in early- and late-phase skin responses (EPSR and LPSR) was observed in the 4×3 nmol administrations, 4 weeks apart group (median % change: EPSR = -39.19% , LPSR = -51.19%) [21].

The merit of this new treatment for HDM was further evaluated in a phase 2b study performed in an EEC; 20 subjects per group received placebo or 4 injections, 4 weeks apart of 3 nmol, 6 nmol, or 12 nmol

of peptides. The 12 nmol group was the best performer, so in the second phase of the study, this group was expanded to 44 subjects and the efficacy of 4 versus 11 administrations (4 weeks apart) were compared with placebo administration. Treatment with 4×12 nmol HDM peptide mixture resulted in a mean change from baseline in the TRSS at 1 year after study initiation of -5.82 compared with a mean change in the placebo group of -3.04 ($p < 0.05$). Global assessment of symptoms and wellbeing also improved significantly. There was no increase in efficacy with the administration of 11 doses over 4 doses, implying that there is not a linear correlation between number of doses and clinical improvement [22].

In a follow-up of 72 of the original 172 randomized subjects, efficacy at approximately 2 years after the initiation of the trial was evaluated in the EEC. Subjects also recorded daily rhinoconjunctivitis symptom scores in the field for a period of 2 weeks after the EEC challenge. Two years after the initiation of the study, 4×12 nmol HDM peptides improved total rhinoconjunctivitis symptom scores in the EEC by up to 33 % compared to placebo. Symptom scores in the field analysis were also improved by 35.8 %. Nasal symptoms scores in both the EEC and field settings improved by similar margins over placebo [23]. In a related sub-study, the safety of HDM peptide immunotherapy was assessed in subjects with HDM-induced allergic rhinoconjunctivitis and Global Initiative for Asthma (GINA) step 1 ($n = 17$) or GINA 2 ($n = 13$) controlled asthma. The randomized subjects receiving 4×12 nmol HDM peptides every 4 weeks were compared to subjects receiving placebo. The results showed that the treatment was also well tolerated by the asthmatic population, with no treatment-related adverse events and similar mean FEV₁ before and after dosing [24].

Grass allergy

Treatment of grass pollen allergy has also been evaluated in a phase 2b clinical trial of peptide immunotherapy with a mixture of seven peptides from grass pollen allergens, three conserved in group 1 allergens and four conserved in group 5 allergens. The sequences employed are representative of a range of different grass species including rye, timothy, Kentucky bluegrass, orchard, velvet, canary, and Bermuda grasses. The study was conducted 4 to 5 months before the start of the grass pollen season. Two hundred and eighty two subjects were randomized to receive placebo or one of three different peptide dosing regimens (8×6 nmol, 2 weeks apart; 4×12 nmol, 1 month apart with placebo infill to complete a 2-weekly injection regimen; 8×12 nmol, 2 weeks apart). Efficacy was measured approximately 25 weeks from the initiation of treatment (after the

Tab. 1: Summary of relevant clinical trials involving peptide-based immunotherapy in allergy

Reference	Allergen	Number of subjects	Study design	Treatment
Norman et al. 1996 [3]	Cat	95 cat allergic subjects	Double-blind, placebo-controlled study	4-weekly subcutaneous injections of IPC-1 and IPC-2 at 2 doses: 7.5, 75, or 750 µg
Haselden et al. 1999 [4]	Cat	40 cat allergic asthmatic subjects	Exploratory study	40 µg intradermal injection of 3 Fel d 1 peptides into each forearm
Simons et al. 1996 [5]	Cat	42 cat allergic subjects	Randomized, double-blind, parallel-group study	4-weekly subcutaneous injections of IPC-1 and IPC-2 (750 µg each)
Pène et al. 1998 [6]	Cat	31 cat allergic subjects	Double-blind, placebo-controlled study	6-weekly subcutaneous injections of 7.5, 75, or 750 µg of Allervax Cat (IPC-1 and IPC-2)
Maguire et al. 1999 [7]	Cat	133 cat allergic subjects	Randomized, placebo-controlled, double-blind study	Twice weekly subcutaneous injections of 75, or 750 µg of Allervax Cat (IPC-1 and IPC-2) for 2 weeks, repeated 4 months later
Muller et al. 1998 [9]	Bee	5 bee venom allergic subjects	Exploratory study	Weekly subcutaneous injections of three peptides derived from Api m 1, totalling up to 400 µg of each peptide
Tarzi et al. 2006 [10]	Bee	12 bee venom allergic subjects	Open label, controlled study	9-weekly intradermal injections of PLA2 peptides to a cumulative dose of 430 µg
Oldfield et al. 2001 [11]	Cat	24 cat allergic subjects	Open dose-ranging study	Intradermal injections of 1, 2.5, and 5 µg peptide-mixtures
Oldfield et al. 2002 [12]	Cat	24 cat allergic subjects	Placebo-controlled, double-blind study	Intradermal injections of 5, 10, 25, and 50 µg (total dose 90 µg) of peptides at 3–4 day intervals over 2 weeks
Alexander et al. 2005 [13]	Cat	16 cat allergic asthmatic subjects	Randomized, placebo-controlled, double-blind study	Intradermal injections of approximately 300 µg of peptides in increasing, divided doses at 2 weeks intervals
Worm et al. 2011 [17]	Cat	88 cat allergic subjects	Phase 2a, double-blind, placebo-controlled trial; escalating dose cohort design	Single intradermal injection of 0.03, 0.3, 3, or 12 nmol Cat-PAD or single subcutaneous injection of 0.03, 0.3, 3, 12, or 20 nmol Cat-PAD
Worm et al. 2013 [18]	Cat	121 cat allergic subjects	Phase 2b environmental exposure chamber (EEC) study	Placebo or one of the following 4 regimens with Cat-PAD: 4 injections x 3 nmol, 2 weeks apart; 4 injections x 6 nmol, 2 weeks apart, 4 injections x 3 nmol, 4 weeks apart; 8 injections x 3 nmol, 2 weeks apart
Patel et al. 2013 [19]	Cat	202 cat allergic subjects	Randomized, placebo-controlled study using EEC	Placebo (8 injections) or Cat-PAD: 8 injections x 3 nmol, 2 weeks apart, or 4 injections x 6 nmol, 4 weeks apart
Couroux et al. 2015 [20]	Cat	51 cat allergic subjects	Randomized, placebo-controlled study using EEC	Initial course of treatment from parent study [19] with 2 year follow up
Larche et al. 2013 [21]	HDM	50 HDM allergic subjects	Phase 2a	4 intradermal administrations, 4-weeks apart of 0.03, 0.3, 1.0, 3.0, and 12 nmol of peptides
Hafner et al. 2014 [22]	HDM	80 HDM allergic subjects	Phase 2b	Placebo or 4 injections, 4 weeks apart of 3 nmol, 6 nmol, or 12 nmol of peptides with 1 year follow-up EEC challenge
Hafner et al. 2015 [23]	HDM	132 HDM allergic subjects	Phase 2b	4 vs. 11 administrations of 12 nmol peptide, 4 weeks apart
Pawsey et al. 2015 [24]	HDM	Follow-up of 72 of the original 172 randomized subjects	Randomized, placebo-controlled study	EEC allergen challenge after 2 years of initial start of trial
Ellis et al. 2016 [25]	Grass	282 grass allergic subjects	Randomized, placebo-controlled study	Placebo or one of three different peptide regimens: 8 x 6 nmol, 2 weeks apart, 4 x 12 nmol, 1 month apart with placebo infill; 8 x 12 nmol, 2 weeks apart
Hafner et al. 2012 [26]	Ragweed	275 ragweed allergic subjects	Randomized, placebo-controlled Phase 2b study	Placebo or one of four treatment regimens

ALLERVAX CAT = synthetic cat allergen peptides; Cat-PAD = cat peptide antigen desensitization; CPT = conjunctival provocation test; EEC = environmental exposure chamber; HDM = house dust mite; IPC-1 and IPC-2 = two 27-amino acid peptides from chain 1 of Fel d 1; PD20FEV1 = provocative dose that induces a 20 % fall in FEV1; PLA2 = Phospholipase A2; TRSS = total rhinoconjunctivitis symptom score

Efficacy	Adverse effects
The higher dose, 750 µg, showed clinical efficacy with reduced baseline nasal and lung scores.	Adverse respiratory events were evident upon administration of 750 µg dose for up to 3 hours after treatment.
Repeat administration of peptides attenuated the late asthmatic reactions.	Isolated late asthmatic reactions in 9/40 subjects.
Failure to reduce skin test reactivity to cat extract.	Treated subjects had more adverse events than placebo-treated subjects.
No improvement in PD20FEV1.	No severe adverse events were reported.
High-dose (750 µg) Allervax Cat showed efficacy in improving pulmonary functions.	9/53 subjects reported severe adverse reaction to Allervax cat treatment.
All subjects tolerated intradermal injection challenge with Api m 1. Three subjects tolerated a bee sting challenge without clinical symptoms while the other two patients experienced mild systemic reactions.	No severe adverse events were reported.
Reduced late-phase skin reaction to intradermal allergen challenge; reduced PBMC production of Th1 and Th2 cytokines; increased IL-10 production.	No severe adverse events were reported.
Peptide-specific bronchial hyporesponsiveness; reduced late-phase skin reaction to allergen challenge.	No severe adverse events were reported.
Reduction in late-phase skin reaction.	No severe adverse events were reported.
Reduction in late asthmatic reaction and improvement in asthma quality of life scores.	No severe adverse events were reported.
Intradermal route was preferred as it showed greater reduction of late-phase skin reactions.	No severe adverse events were reported and injections were tolerated at high doses.
Group with 8 injections × 3 nmol, 2 weeks apart had greatest decrease in mean change in TRSS.	No serious adverse events were reported and other adverse events occurred in both the placebo and active groups.
Treatment with the 4 × 6 nmol regimen showed greatest efficacy that persisted 1 year after the start of treatment.	Severe adverse event reported in the placebo group and not related to drug.
Improved TRSS with 4 × 6 nmol regimen was persistent after 2 years from start of treatment.	No severe adverse events were reported.
Reduced CPT response in all four treatment arms and largest change in early- and late-phase skin responses in the 4 × 3 nmol group.	Safe and well tolerated with no serious adverse events.
12 nmol showed efficacy and was the selected dose for the following study [23].	No severe adverse events were reported.
4 × 12 nmol HDM decreased TRSS and there was no increase in efficacy with 11 administrations. The reduction in symptoms persisted 1 year after the start of treatment.	No severe adverse events were reported.
4 × 12 nmol HDM peptides improved total rhinoconjunctivitis symptom scores; Nasal symptom scores improved in the EEC and field settings.	Treatment was well tolerated with no treatment-related adverse events.
Greatest efficacy was seen with the group that received 8 × 6 nmol, 2 weeks apart, with decreased TRSS and 44 % of subjects considering themselves as "much improved".	No severe adverse events were reported.
Treatment with highest dose of peptides displayed decrease in TRSS at post-treatment EEC challenges.	Treatment was safe and well tolerated.

peak of the grass pollen season) using an Environmental Exposure Unit (EEU; Queen's University/Kingston General Hospital, Ontario, Canada). Grass allergic subjects were exposed to rye grass allergen that was distributed into the EEU by dispersion to achieve consistent levels of exposure (mean hourly range of $3,500 \pm 500$ grains/m³). Exposure occurred for 3 hours per day for 4 consecutive days with TRSS measured at 30 minute intervals. The primary endpoint of the study was the change from baseline challenge in the mean TRSS at all time points on days 2–4 of the post-peptide treatment EEU exposure in subjects receiving grass peptide immunotherapy vs. placebo at 25 weeks after treatment initiation. Exploratory endpoints included rye-grass-specific IgA, IgE, and IgG4 measurements before and after treatment. The greatest level of efficacy was observed in the group that received 8×6 nmol (2 weeks apart) with a mean change in TRSS from baseline of -5.4 compared to -3.8 in the placebo group (a 42% improvement over placebo; $p < 0.05$). Furthermore, a subjective global assessment of symptom improvement showed that 44% of subjects treated with the 6 nmol regimen considered themselves to be “much improved” after treatment, compared to 22% of subjects in the placebo group ($p < 0.01$) [25].

In an optional extension of the same study, subjects achieving a mean baseline TRSS of ≥ 8 attended for a repeat 4 day EEU pollen exposure at approximately 1 year after initiation of peptide dosing (i.e., following two grass pollen seasons). Subjects who had received the 8×6 nmol regimen continued to exhibit improved mean TRSS (-6.0) compared to subjects receiving placebo (-3.6 ; $p = 0.0535$). Interestingly, the reduction in mean TRSS for the 6 nmol group was greater than that in two other treatment groups receiving higher doses of peptides.

Ragweed allergy

Peptides from the major ragweed allergen Amb a 1 were used to treat ragweed allergic subjects in double-blind, placebo-controlled phase 2b study evaluating TRSS in an EEC setting. Two hundred and seventy five subjects were randomized to receive placebo or one of four ragweed peptide treatment regimens. The largest changes in TRSS were observed in the most symptomatic subjects. Treatment with the highest dose of peptides showed a mean change from baseline in TRSS scores at the post-treatment EEC challenges (conducted at 18–22 weeks after study initiation) of -5.77 vs. -2.93 in the placebo group ($p < 0.05$) [26].

Taken together, these studies in environmental chamber models demonstrate the efficacy and tolerability of the peptide immunotherapy approach for the treatment of allergic rhinitis. The next step

will be confirmation of these results in a larger (phase 3) clinical trial and further evaluation of efficacy in subjects with allergic asthma.

Mechanisms of action

The mechanism of actions of whole allergen immunotherapy (AIT) [27] is thought to rely on the production of allergen-specific IgG1 and IgG4 (and in some studies IgA) “blocking” antibodies, and on the down-regulation of specific T cell responses against the allergen, mainly ascribable to induction of Treg [28], increased production of IL-10 [29, 30], and/or shift of the Th1/Th2 ratio [31, 32]. In the case of peptide immunotherapy, how peptides achieve clinical efficacy remains only partially understood, although evidence suggests that at least some of the candidate mechanisms overlap with the whole allergen approach (Fig. 2).

Treatment of allergic individuals with long peptides (contiguous overlapping peptides, COP) from either bee venom phospholipase A2 or birch pollen Bet v 1 has been shown to induce allergen-specific IgG antibodies which is undoubtedly related to the size of the peptides (> 40 amino acids) and the presence of conformational B cell epitopes allowing B cell receptor cross-linking. Induction of allergen-specific IgG4 was also demonstrated. However, the use of these long peptides was associated with frequent adverse events including systemic reactions [15, 16].

Although short peptides may contain linear B cell epitopes, their monomeric delivery to the immune system, their lack of tertiary structure, and inability to cross-link B cell surface antigen receptors make it unlikely that they would be able to drive antibody production (blocking antibody or IgE) directly. In studies of short peptide immunotherapy that have reported allergen-specific IgG levels before and after peptide immunotherapy, weak or absent increases have been observed. Ellis and colleagues reported grass allergen-specific IgG4, IgA, and IgE levels in 179 grass allergen peptide-treated individuals [25]. No significant changes were observed after peptide treatment, despite exposure to whole allergen (during a pollen season) containing conformational B cell epitopes. Interestingly, although only two studies have been published reporting the results of short peptide immunotherapy for bee venom allergy, both were associated with the induction of detectable allergen-specific IgG4 responses. Of note, exposure to whole allergen in these studies (injections of allergen extract were used as outcome measures in both studies) may have been higher than in the grass allergen peptide-treated individuals [9, 10]. Thus, it maybe that immunotherapy with short peptides does not directly induce blocking antibodies, but that follow-

ing subsequent exposure to sufficient whole allergen protein, allergen-specific blocking antibodies may be induced and may contribute to efficacy. This notion requires further investigation.

Much of the accumulated evidence on the mechanisms of action of peptide immunotherapy in allergy derives from early clinical studies and from findings in animal models [10, 11, 12, 13, 33, 34, 35]. These studies point towards modulation of the allergen-specific T cell response and the establishment of a CD4+ T cell population with suppressive capacity, although the exact phenotype and characteristics of such a population remains unclear. Several clinical studies have reported increased production of IL-10 from cultures of PBMC stimulated with allergen after peptide immunotherapy [10, 12, 34]. Murine models also emphasize a potential role for IL-10 in peptide immunotherapy. Moldaver and colleagues demonstrated the induction of bystander tolerance (inter-molecular) to ovalbumin following treatment with house dust mite peptides. Tolerance was associated with increased production of

IL-10 and increased proportions of FoxP3+ cells making IL-10 [36].

Campbell and colleagues investigated mechanisms of peptide-induced tolerance in both murine and human systems. In mice, peptide treatment was associated with suppression of Th2 cytokine production and with a marked increase in the frequency of CD4+ T cells secreting IL-10; indeed, the numbers of T cells secreting IL-10 was greater than could be accounted for by allergen-specific T cells (established using MHC class II tetramers) and thus provided further evidence of bystander tolerance. Reduced clinical outcomes in this model were blocked with the administration of anti-IL-10 monoclonal antibodies immediately after treatment with the peptide, suggesting a correlation between IL-10 and efficacy. As part of the same study in vitro responses of pre- and post-treatment PBMC were measured following culture with allergen and individual peptides comprising not only peptides used as part of the treatment, but also others from the same allergen that were not administered therapeutically. The

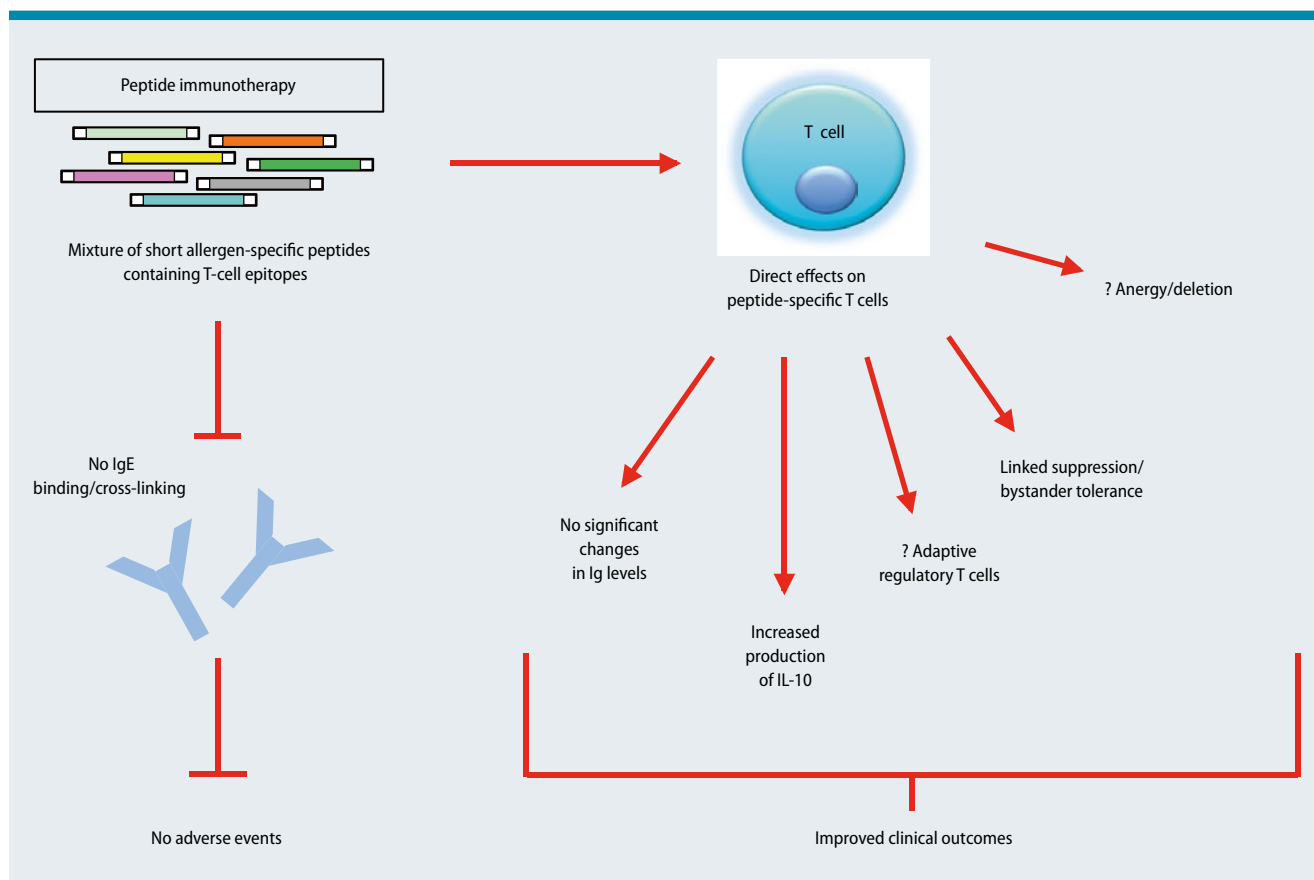


Fig. 2: Proposed mechanisms of action of peptide immunotherapy. The treatment involves intradermal administrations of a mixture of peptides containing multiple dominant T cell epitopes. These peptides are physically unable to cross-link IgE and release histamine. Upon presentation, they induce direct effects on rare epitope-specific T cells that lead, through systemic mechanisms still not completely understood, to the improvement of clinical symptoms.

results provided evidence of linked epitope suppression (intra-molecular tolerance) since in addition to suppression of proliferative and cytokine responses directed against the treatment peptides, similar reductions were observed to non-treatment peptides demonstrating that treatment with certain T cell epitopes of a protein can also induce tolerance to other epitopes. The phenomena of bystander tolerance and linked epitope suppression hold potential promise for the exploitation of peptide-induced tolerance to improve clinical efficacy [33].

Future perspectives

Currently available data suggest that peptide immunotherapy provides long-lasting efficacy following a short treatment course. As no studies have followed treated subjects beyond two years, it is possible that efficacy endures beyond this period and it is conceivable that the treatment may be permanently curative. Future research will likely explore combining peptides with adjuvants or other compounds that may facilitate improved efficacy or reduce the dose of peptides required. Mechanisms of action remain incompletely understood, but clarification of molecules and pathways modulated by peptide immunotherapy will provide insight, not only into the mechanisms of action of the intervention itself, but also into the pathogenesis of allergic reactions.

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Conflict of interest

Mark Larché is a co-founder of Circassia Ltd., is a current consultant to the company and a shareholder in Circassia Pharmaceuticals plc. Mark Larché is a consultant to Adiga Life Sciences Inc. Mark Larché has received research development contracts from both Circassia and Adiga Life Sciences. Mark Larché is a named inventor on patents held by Circassia Ltd. In the last 12 months Mark Larché has also acted as a paid consultant to UCB and consults for Aravax Pty. Elena Tonti has received salary support and research grant support from Adiga Life Sciences Inc.

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