

Immunotherapy of the futur

Allergen-derived T cell peptides in immunotherapy

Les peptides T en immunothérapie

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Abstract

The incidence of allergic diseases has continued to rise during the last 30 years. A combination of genetic and environmental factors is likely to be responsible for the increased prevalence of both allergic and autoimmune diseases. Whilst the majority of approaches towards treatment are palliative, specific allergen immunotherapy (SIT) is an approach that has been proven to be efficacious and disease-modifying. However, as a result of interaction between allergen-specific IgE and conformational B cell epitopes on the surface of the allergen molecule, SIT is associated with adverse reactions during treatment. One strategy developed to reduce IgE-mediated adverse events whilst maintaining clinical efficacy has been to treat subjects with short peptide sequences corresponding to T cell epitopes of the allergen. The size and the monomeric nature of peptides have allowed relatively large doses of antigen to be delivered in soluble form. Treatment with peptides has been shown to improve clinical surrogate measurements of disease, to modify the immune response to allergen and to improve patients' perception of disease. This review will summarise results achieved in recent years with peptide immunotherapy and discuss the mechanisms that may be responsible for efficacy.

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Résumé

L'incidence des affections allergiques a augmenté au cours des 30 dernières années. L'association de facteurs génétiques et environnementaux est probablement responsable de l'augmentation de prévalence des affections allergiques et auto-immunes. Alors que la plupart des approches thérapeutiques sont palliatives, l'immunothérapie spécifique (ITS) a prouvé son efficacité et modifie l'histoire naturelle de l'allergie. Cependant, conséquence de l'interaction entre les IgE spécifiques de l'allergène et les épitopes des cellules B à la surface de la molécule allergénique, l'ITS est associée à des effets thérapeutiques adverses. Une stratégie visant à diminuer ces effets adverses IgE-dépendants, tout en maintenant l'efficacité clinique, est d'utiliser de courtes séquences peptidiques correspondant aux épitopes des cellules T. La taille et la nature monomérique de ces peptides permet d'employer des doses assez importantes d'antigène sous forme soluble. Le traitement par les peptides a amélioré les paramètres cliniques de l'affection, modifié la réponse immune à l'allergène, et amélioré la perception clinique de l'affection par les patients. Cette revue résume les résultats obtenus au cours des dernières années avec l'immunothérapie utilisant les peptides et discute les mécanismes qui peuvent être responsables de cette efficacité.

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Keywords: Peptide; Epitope; T cell; Lymphocyte; Allergy; Immunotherapy**Mots clés :** Peptide ; Épitopes ; Cellules T ; Lymphocytes ; Allergie ; Immunothérapie spécifique**1. Introduction**

Specific whole allergen immunotherapy (specific immunotherapy; SIT) has been shown to be highly efficacious in

carefully selected patients both with allergic rhinitis [1] and with asthma [2]. However, due to the use of extracts containing native allergen molecules capable of cross linking allergen-specific IgE, SIT is associated with a relatively high incidence of adverse events. The frequency and severity of such events limit the dose that can be administered to patients and result in a protracted course of treatment.

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The mechanisms underlying the success of SIT are incompletely understood. However, modulation of allergen-specific T cell responses appears to occur such that a relative increase in Th1 responses to allergen are often observed. There is also some evidence that a regulatory IL-10 phenotype may also be induced in some forms of SIT [3,4]. In order to maintain the efficacy of immunotherapy whilst reducing IgE-mediated adverse events, the use of short linear peptides has been proposed. Such preparations should comprise known T cell epitopes of the allergen but should contain insufficient secondary structure to allow cross linking of IgE molecules on the surface of effector cells such as mast cells and basophils.

2. Experimental models of immunological tolerance

A number of groups have demonstrated the efficacy of peptides in the prevention and/or treatment of disease in animal models of autoimmunity and allergy. Peptide-induced tolerance has been demonstrated in models of experimental autoimmune encephalomyelitis (EAE; [5,6]), collagen-induced arthritis [7], diabetes [8] and systemic lupus erythematosus (SLE) [9]. Briner and colleagues demonstrated the ability of allergen-derived peptides to inhibit T cell cytokine and antibody production to the cat allergen Fel d 1 in sensitised mice [10]. Hoyne used peptides from the house dust mite allergen Der p 2 to prevent sensitisation by prior vaccination and to down regulate T cell and antibody responses to intact protein [11,12]. A dominant T cell epitope of Bet v 1 was used in CBA/J mice either prior to, or following sensitisation with the whole allergen. T cell proliferative responses were inhibited by both treatments [13]. Mice sensitised to bee venom allergen phospholipase A₂ (PLA₂), were given a mixture of three long peptides spanning the entire PLA₂ molecule by intranasal administration. A decreased IL-4:IFN γ ratio was observed coupled with a marked reduction in specific IgE. Pre-administration of peptide resulted in a failure to develop IgE sensitisation to subsequent immunisation with the whole molecule [14].

High doses of peptides have been employed in *in vitro* studies to modulate human T cell responses. Lamb and colleagues demonstrated the induction of antigen-specific non-responsiveness following exposure of T cell clones to supraoptimal doses of cognate ligand in the absence of professional antigen-presenting cells [15]. Collectively, these studies provided a logical basis for the translation of a peptide-based therapeutic approach in man.

3. Clinical studies evaluating allergen-derived peptides

Human studies evaluating peptide epitope therapy have begun to emerge over the past 7 years. Results have been mixed as would be expected with the advent of a novel approach. The majority of the data has been generated from

patients allergic to cats. Treatment with various formulations of peptides has been evaluated. Norman and colleagues [16] administered four injections of Fel d 1 peptides (residues 7–32 and 29–55 of chain 1) or placebo to 95 cat-sensitive patients in three dose groups (7.5 μ g, 75 μ g and 750 μ g per injection). Both nasal and lung symptom scores improved in response to peptide therapy in the high dose group, although a prominent placebo effect was observed. Treatment was associated with a significant incidence of adverse events, which occurred a few minutes to several hours after peptide injection. Further studies [17] demonstrated reduced IL-4 production in IPC-1/IPC-2-specific T cell lines following therapy.

Pène and colleagues [18] also reported a reduction in IL-4 production in PBMC challenged with allergen before and after treatment with IPC-1/IPC-2. A significant reduction (compared with background levels) of IL-4 production was observed in the high dose group (individual dose 750 μ g; total dose 1500–4500 μ g) but not in the lower dose groups. A reduction in allergen PD₂₀ was also observed in response to both high (1500–4500 μ g) and medium (individual dose 75 μ g; total dose 150–450 μ g) dose regimens when compared with baseline (within group), but not when compared with placebo (between group). The variability in dose between each group arose as a result of a staggered dose protocol in which a number of individuals received weekly injections for 2, 4 or 6 weeks.

A double-blind, parallel group study administering weekly injections (over 4 weeks), of 250 μ g of IPC-1 and IPC-2 to 42 (21 active and 21 placebo) individuals with cat-induced rhinitis and/or asthma was performed by Simons and colleagues [19]. Late onset symptoms of rhinitis, asthma and pruritis were observed following treatment. No change in early and late phase skin responses to whole allergen were recorded at 2, 6 and 24 weeks after treatment. PBMC cytokine secretion profiles remained unaltered after therapy.

A multi-centre study of 133 subjects receiving eight injections of 750 μ g IPC-1/IPC-2, demonstrated an improvement in pulmonary function in those subjects who had diminished baseline FEV1. However, improvements were evident at only a single time point (3 weeks) post therapy [20]. A total of 6 mg of each peptide was administered in two blocks of four injections. Frequent adverse events were reported including some that required the use of epinephrine. Like other studies evaluating IPC-1/IPC-2, late onset adverse reactions diminished with successive doses of peptide suggesting the induction of hypo-responsiveness.

Early adverse events described in the studies above may have been due to cross linking of IgE by IPC-1 and IPC-2 which, at 27 residues each, were larger than an average MHC class II-restricted T cell epitope (approximately 15 residues). Other, more common adverse events were characterised by symptoms of breathlessness and wheeze with an onset several hours after peptide administration. Studies in our own group have confirmed that these symptoms were probably T

cell-dependent, IgE-independent, isolated late asthmatic reactions (LAR) [21].

Norman and colleagues reported a reduction in late adverse events with successive peptide administration compatible with the induction of hypo-responsiveness or peripheral T cell tolerance [16]. Oldfield induced LAR in cat allergic asthmatics and then re-challenged them several weeks after their initial reaction. The second challenge failed to induce LAR unless it was administered several months after the first. Based on data derived from several subjects receiving peptide injections with different intervals, it was possible to demonstrate a “window” of hypo-responsiveness that occurs at approximately 2–8 weeks after a single peptide injection [22]. From 8 weeks on, the ability to react to peptide challenge gradually returns but takes 4–6 months to reach baseline. It may be that a series of injections of increasing peptide dose may extend the refractory period and result in protection from re-challenge for a period of a year or more.

In the same study the effect of peptide administration on responsiveness to whole allergen challenge was evaluated. Cat allergic asthmatics were challenged intradermally with whole cat dander allergen extract, before and after a single injection of 5 µg of each of 12 peptides in a mixture. The 12 peptides (termed MOP; multiple overlapping peptides) spanned the majority of the Fel d 1 molecule in order to provide T cell epitopes for the majority of the population. Following a single peptide injection, the magnitude of the cutaneous late phase response to allergen was markedly reduced. Some subjects developed isolated LARs following peptide injection, whereas others did not. LARs were not obligatory for the reduction in cutaneous responses implying that the two mechanisms were not linked.

The clinical efficacy of multiple incremental doses of MOP was recently evaluated in cat allergic asthmatics [23]. Twenty-four cat allergic asthmatic subjects with moderate to severe asthma were enrolled and subjected to cutaneous allergen challenge, inhaled methacholine PC₂₀ and PD₂₀ together with quality of life evaluated by questionnaire. Subjects were treated with a total of 90 µg of each of 12 peptides (MOP; each of 16–17 amino acids in length in saline). Doses were administered at 3–4 day intervals. As the subjects were asthmatic, it was expected that approximately 50% of those on active treatment would develop peptide-induced, isolated late asthmatic reactions (LAR; characterised by a 20% or greater fall in FEV₁) during peptide up dosing (based on previous observations). In this eventuality, repeat injections of the same dose were administered until changes in FEV₁ during the 6 h period after injection were less than 20% from baseline. At this point, up dosing was reinstated with adjustments made in order to deliver the same cumulative dose to all subjects.

Subjects underwent two follow-up assessments after treatment at which baseline parameters were repeated. The first follow-up was 4–8 weeks after therapy and the second, 3–9 months. A total of eight subjects on active treatment experienced LAR following the first or subsequent peptide injections.

In each case the magnitude of the reaction diminished on successive injections. Two of the placebo group also experienced a reduction in FEV₁ of greater than 20% emphasising the moderate to severe nature of the disease in the group as a whole.

The magnitude of both early and late phase cutaneous reactions to intradermal challenge with the purified allergen Fel d 1 were significantly reduced following peptide therapy. Cutaneous reactions to whole cat dander allergen extract were also significantly reduced from baseline in treated patients. Reduction in the magnitude of early phase reactions was not evident at the first (4–8 week) follow-up, implying a dissociation between the modulation of the IgE-mediated EPR and the largely T cell-driven LPR which was reduced at the 4–8 week follow-up and further reduced at the later time point.

No change was observed in either the allergen PC₂₀ or the histamine PC₂₀ measurements following treatment. However, the study was not powered to detect significant changes in these parameters. More recently, in a small open study, administration of peptides at 2 weekly intervals resulted in an improvement in histamine PC₂₀ [24]. Analysis of allergen-specific peripheral blood mononuclear cell (PBMC) responses *in vitro* demonstrated reductions in proliferation and pro-inflammatory cytokines (IL-4, IL-13, IFNγ). In agreement with findings in a number of animal models of peptide therapy in chronic diseases, IL-10 production was elevated at both follow-up time points. Reduction of pro-inflammatory cytokines together with a concomitant rise in IL-10 suggests the induction of active immunoregulation following peptide treatment.

Muller and colleagues treated five subjects allergic to bee venom, with a mixture of three peptides from the major venom allergen phospholipase A₂ [25]. Patients had experienced a previous moderately severe systemic reaction following wild bee sting. Peptides were administered at weekly intervals in an up dosing fashion. A total dose of 397.1 µg was administered. After therapy, all five subjects tolerated intradermal challenge with 10 µg of purified PLA₂. Following a wild bee sting in the clinical laboratory, three subjects experienced no reaction whilst the remaining two experienced mild localised reactions at the sting site. T cell responses to both peptides and whole allergen were evaluated *in vitro*. The induction of clinical hyporesponsiveness was accompanied by reductions in T cell proliferative responses and inflammatory cytokines.

4. Mechanisms in immunotherapy

Analysis of the mechanisms involved in successful whole allergen immunotherapy have described a shift in T helper cytokine phenotype from Th2 towards Th1 (reviewed by Rolland and O'Hehir [26]). Most commonly, an increase in the ratio of IFNγ:IL-4 was observed together with a concomitant change in the proportions of allergen-specific IgG

to IgE. However, in certain models of SIT (particularly venom therapy), treatment is accompanied by the induction of a regulatory profile of cytokine production characterised by the presence of IL-10.

Akdis and colleagues provided evidence for a role for IL-10 in establishing PLA₂-specific hyporesponsiveness in allergic individuals undergoing successful immunotherapy (whole allergen or peptide) and also in hyperimmune bee keepers [27,28]. Nasser and colleagues found increased levels of IL-10 mRNA and protein in skin biopsies from allergen-challenged subjects following wasp venom immunotherapy [3]. Our own studies with peptides derived from Fel d 1 suggest that whilst pro-inflammatory cytokines are decreased after peptide therapy, IL-10 production is markedly increased [23]. Together, these findings suggest that peptide immunotherapy may work through the induction of immune regulation characterised by enhanced IL-10 production and concomitant suppression of pro-inflammatory cytokines of both Th1 and Th2 classes.

5. Conclusions

Antigen-specific immunotherapy can modify disease processes without affecting responses to unrelated antigens. Peptide immunotherapy, particularly in allergic diseases, may offer the prospect of regulating immune responses in a specific way, with a substantially reduced risk of IgE-mediated adverse events. SIT has demonstrated that antigen-specific modulation is efficacious. However, the risk of IgE-mediated adverse reactions associated with the use of whole allergen molecules has limited this form of therapy. The use of short peptide sequences with reduced IgE cross linking capacity results in safer and more efficacious immunotherapy. Studies identifying T cell epitopes in a number of major allergens are ongoing and provide the prospect of peptide-based vaccines for a wide range of allergic sensitisations.

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References

- [1] Durham SR, Walker SM, Varga EM, Jacobson MR, O'Brien F, Noble W, Till SJ, Hamid QA, Nouri-Aria KT. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999;341:468–75.
- [2] Abramson M, Puy R, Weiner J. Immunotherapy in asthma: an updated systematic review. *Allergy* 1999;54:1022–41.
- [3] Nasser SM, Ying S, Meng Q, Kay AB, Ewan PW. Interleukin-10 levels increase in cutaneous biopsies of patients undergoing wasp venom immunotherapy. *Eur J Immunol* 2001;31:3704–13.
- [4] Akdis CA, Blaser K. Mechanisms of interleukin-10-mediated immune suppression. *Immunology* 2001;103:131–6.
- [5] Clayton JP, Gammon GM, Ando DG, Kono DH, Hood L, Sercarz EE. Peptide-specific prevention of experimental allergic encephalomyelitis. Neonatal tolerance induced to the dominant T cell determinant of myelin basic protein. *J Exp Med* 1989;169:1681–91.
- [6] Gaur A, Wiers B, Liu A, Rothbard J, Fathman CG. Amelioration of autoimmune encephalomyelitis by myelin basic protein synthetic peptide-induced anergy. *Science* 1992;258:1491–4.
- [7] Staines NA, Harper N, Ward FJ, Malmstrom V, Holmdahl R, Bansal S. Mucosal tolerance and suppression of collagen-induced arthritis (CIA) induced by nasal inhalation of synthetic peptide 184–198 of bovine type II collagen (CII) expressing a dominant T cell epitope. *Clin Exp Immunol* 1996;103:368–75.
- [8] Daniel D, Wegmann DR. Protection of nonobese diabetic mice from diabetes by intranasal or subcutaneous administration of insulin peptide B-(9-23). *Proc Natl Acad Sci USA* 1996;93:956–60.
- [9] Wu HY, Ward FJ, Staines NA. Histone peptide-induced nasal tolerance: suppression of murine lupus. *J Immunol* 2002;169:1126–34.
- [10] Briner TJ, Kuo MC, Keating KM, Rogers BL, Greenstein JL. Peripheral T-cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d 1. *Proc Natl Acad Sci USA* 1993;90:7608–12.
- [11] Hoyne GF, O'Hehir RE, Wraith DC, Thomas WR, Lamb JR. Inhibition of T cell and antibody responses to house dust mite allergen by inhalation of the dominant T cell epitope in naive and sensitized mice. *J Exp Med* 1993;178:1783–8.
- [12] Hoyne GF, Askonas BA, Hetzel C, Thomas WR, Lamb JR. Regulation of house dust mite responses by intranasally administered peptide: transient activation of CD4⁺ T cells precedes the development of tolerance in vivo. *Int Immunol* 1996;8:335–42.
- [13] Bauer L, Bohle B, Jahn-Schmid B, Wiedermann U, Daser A, Renz H, Kraft D, Ebner C. Modulation of the allergic immune response in BALB/c mice by subcutaneous injection of high doses of the dominant T cell epitope from the major birch pollen allergen Bet v 1. *Clin Exp Immunol* 1997;107:536–41.
- [14] Astori M, Von Garnier C, Kettner A, Dufour N, Corradin G, Spertini F. Inducing tolerance by intranasal administration of long peptides in naive and primed CBA/J mice. *J Immunol* 2000;165:3497–505.
- [15] Lamb JR, Skidmore BJ, Green N, Chiller JM, Feldmann M. Induction of tolerance in influenza virus-immune T lymphocyte clones with synthetic peptides of influenza hemagglutinin. *J Exp Med* 1983;157:1434–47.
- [16] Norman PS, Ohman JL, Long AA, Creticos PS, Gefter MA, Shaked Z, Wood RA, Eggleston PA, Hafner KB, Rao P, Lichtenstein LM, Jones NH, Nicodemus CF. Treatment of cat allergy with T-cell reactive peptides. *Am J Respir Crit Care Med* 1996;154:1623–8.
- [17] Marcotte GV, Braun CM, Norman PS, Nicodemus CF, Kagey-Sobotka A, Lichtenstein LM, Essayan DM. Effects of peptide therapy on ex vivo T-cell responses. *J Allergy Clin Immunol* 1998;101:506–13.
- [18] Pene J, Desroches A, Paradis L, Lebel B, Farce M, Nicodemus CF, Yssel H, Bousquet J. Immunotherapy with Fel d 1 peptides decreases IL-4 release by peripheral blood T cells of patients allergic to cats. *J Allergy Clin Immunol* 1998;102:571–8.
- [19] Simons FE, Imada M, Li Y, Watson WT, HayGlass KT. Fel d 1 peptides: effect on skin tests and cytokine synthesis in cat-allergic human subjects. *Int Immunol* 1996;8:1937–45.
- [20] Maguire P, Nicodemus C, Robinson D, Aaronson D, Umetsu DT. The safety and efficacy of ALLERVAX CAT in cat allergic patients. *Clin Immunol* 1999;93:222–31.

- [21] Haselden BM, Kay AB, Larche M. Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. *J Exp Med* 1999;189:1885–94.
- [22] Oldfield WL, Kay AB, Larche M. Allergen-derived T cell peptide-induced late asthmatic reactions precede the induction of antigen-specific hyporesponsiveness in atopic allergic asthmatic subjects. *J Immunol* 2001;167:1734–9.
- [23] Oldfield WL, Larche M, Kay AB. Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. *Lancet* 2002;360:47–53.
- [24] Alexander C, Oldfield WL, Shirley K, Larche M, Kay AB. A dosing protocol of allergen-derived T cell peptide epitopes for the treatment of allergic disease. *J Allergy Clin Immunol* 2001;107:716 (abstract).
- [25] Muller U, Akdis CA, Fricker M, Akdis M, Blesken T, Bettens F, Blaser K. Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. *J Allergy Clin Immunol* 1998;101:747–54.
- [26] Rolland J, O'Hehir R. Immunotherapy of allergy: anergy, deletion, immune deviation. *Curr Opin Immunol* 1998;10:640–5.
- [27] Akdis CA, Akdis M, Blesken T, Wymann D, Alkan SS, Muller U, Blaser K. Epitope-specific T cell tolerance to phospholipase A2 in bee venom immunotherapy and recovery by *IL-2* and *IL-15* in vitro. *J Clin Invest* 1996;98:1676–83.
- [28] Akdis CA, Joss A, Akdis M, Blaser K. Mechanism of *IL-10*-induced T cell inactivation in allergic inflammation and normal response to allergens. *Int Arch Allergy Immunol* 2001;124:180–2.