Mechanisms of Peptide Immunotherapy in Allergic Airways Disease

Mark Larché

Department of Medicine, Firestone Institute for Respiratory Health, McMaster University, Hamilton, Ontario, Canada

Abstract

Allergen immunotherapy with whole proteins is clinically efficacious but requires a protracted treatment period because of frequent allergic adverse events. A combination of duration of treatment and adverse events leads to poor compliance. Short synthetic peptides containing the major immunodominant T cell epitopes of allergenic proteins have been shown to reduce IgE cross-linking ability, thereby leading to fewer allergic adverse events following their administration to patients with allergies. Peptide immunotherapy has been shown to result in clinically meaningful efficacy in several Phase II, randomized, double-blind, placebo-controlled clinical trials. Exactly how peptide immunotherapy achieves its efficacy remains incompletely understood, but the mechanisms are thought to include immune deviation and induction of regulatory T cells capable of suppressing allergen-specific immune responses. Limited data are available on the effects of peptide therapy on humoral immune responses. Induction of allergen-specific IgG has been observed after peptide therapy, but the levels of antibody induced were much lower than generally seen with the utilization of whole allergen approaches. Thus, the immunological mechanisms of peptide immunotherapy appear to overlap, although not completely, with those seen in whole allergen therapy. Further studies are required to fully elucidate mechanisms of action.

Keywords: allergy; epitope; immune tolerance; immunology; T cell

(Received in original form February 28, 2014; accepted in final form May 26, 2014)

Supported by the Canada Research Chairs Program the Canadian Institutes of Health Research, the National Institute of Allergy and Infectious Diseases, the Immune Tolerance Network, the AllerGen Network Centre of Excellence, the Canada Foundation for Innovation, and Boehringer Ingelheim.

Correspondence and requests for reprints should be addressed to Mark Larché, Ph.D., Department of Medicine, Firestone Institute for Respiratory Health, McMaster University, HSC 4H20, 1280 Main Street West, Hamilton, ON, L8S 4L8 Canada. E-mail: larche@mcmaster.ca

Ann Am Thorac Soc Vol 11, Supplement 5, pp S292–S296, Dec 2014

DOI: 10.1513/AnnalsATS.201402-090AW
Internet address: www.atsjournals.org

Currently available treatment for allergic diseases is focused predominantly on pharmacotherapy that addresses symptoms but not pathogenesis. Disease-modifying therapy has been available for more than 100 years in the form of specific allergen immunotherapy (SIT) (1). SIT has been widely demonstrated to be clinically effective and to provide amelioration of symptoms for periods well in excess of the treatment period (2, 3). Prolonged efficacy suggests a fundamental modification of the underlying disease process, which may be regarded as the induction of "tolerance" to the sensitizing allergen. The immunological mechanisms underlying this process remain incompletely understood. Furthermore, the recent emergence of sublingual immunotherapy (SLIT), in addition to the conventional subcutaneous immunotherapy (SCIT), has resulted in further mechanistic complexity because there are clearly subtly different mechanisms associated with the induction of tolerance at mucosal surfaces (4). Despite the favorable duration of efficacy, therapy with whole allergen extracts (or, indeed, with recombinant whole allergens) requires an extended period of treatment, with the accepted optimal period being 3 years. The need for such a long treatment period arises as a result of adverse events, predominantly IgE-mediated, that result from the interaction of the patient's allergen-specific IgE with the treatment allergen. Adverse events range from mild local reactions to severe systemic reactions, including anaphylactic shock that occasionally results in death. In recent studies, investigators have highlighted the impact of adverse events and protracted treatment on compliance, with researchers in one study suggesting that <20% of patients complete a 3-year course of treatment (5). On the basis of this current knowledge, though it appears that allergen immunotherapy has the potential to substantially reduce the physical and economic burden of allergic disease, the nature of approaches using whole allergens prevents wider uptake of this therapy. Thus, there remains an important unmet clinical and economic need for safer, shorter therapies that will address the current technology gap.

In principle, removal of IgE epitopes from whole allergens has the potential to

reduce allergic adverse events. A variety of methods to achieve this end has been developed, including the use of short synthetic peptides that constitute the major T cell epitopes of the allergen. Other peptide-based approaches under development involve the use of B cell epitopes, which are thought to drive a protective IgG response to the whole allergen (6). Synthetic peptides have a number of advantages over the current use of allergen extracts: They can be manufactured under standardized conditions, are easy to purify and are stable in lyophilized form.

In this review, I describe clinical and mechanistic studies undertaken with allergen-derived T cell epitope peptides administered through the intradermal route for the treatment of allergic airways disease. Some of the results of these studies have been previously reported in the form of abstracts (7, 8).

Clinical Efficacy of Peptide Immunotherapy

The clinical efficacy of peptide immunotherapy has been demonstrated in a series of Phase IIb, randomized, double-blind, placebo-controlled clinical trials performed in environmental exposure chambers (EECs) and/or environmental exposure units (EEUs) in which subjects were exposed to controlled levels of circulating airborne allergen. Volunteers allergic to cats were treated with a mixture of seven peptides representing immunodominant epitopes of the major cat allergen Fel d 1. Baseline symptoms elicited by a 4-day exposure to controlled levels of cat allergen were compared with an identical exposure 4 to 5 months later (posttherapy). Symptoms, as measured on a 24-point Total Rhinoconjunctivitis Symptom Score (TRSS), were reduced following delivery of eight intradermal injections of a 3 nM dose of peptide formulation referred to as Cat-PAD (9). In a similarly designed study, researchers subsequently evaluated changes in TRSS in the EEC model following four administrations of a higher dose (6 nM). Similar results were observed at the 18- to 22-week post-treatment follow-up. Additionally, identical EEC evaluations at 50–54 weeks post-treatment demonstrated significant reduction in TRSS compared with the group treated with placebo, despite 9 months without treatment (10). In a protocol extension with blinding maintained, volunteers were also evaluated at the 2-year time point (100–104 wk) by EEC exposure. Although the numbers of subjects were reduced in this voluntary extension study, TRSS symptom scores remained suppressed at a level similar to the 1-year follow-up time point (unpublished data).

Interestingly, there appeared to be a threshold dose effect with respect to efficacy, such that at 50 to 54 weeks and 100 to 104 weeks, a 6 nM dose administered four times was substantially more effective than the equivalent cumulative dose consisting of eight injections at 3 nM, which did not achieve a statistically significant response at these time points. Median TRSS scores adjusted for baseline were similar at the earlier time point of 18 to 22 weeks, however. The dose–response relationship for SCIT has been described as "sharp" (11), referring to the fact that a relatively modest change in dose can have marked effects on clinical response. In reality, it may be that a threshold dose of peptide, or allergen, is required to achieve the full expression of immune tolerance. Lower doses may have a transient clinical benefit, as described above, that wanes with time.

In all of these previous studies, adverse events in the active treatment groups were similar to placebo. These data confirm the enduring clinical efficacy of peptide immunotherapy and point to mechanisms associated with the induction of immunological tolerance. Most recently, Phase IIb results have also been reported for peptide immunotherapies for house dust mite (HDM) allergy and grass allergy. The results, which were published in abstract form, suggest that similar efficacy and safety are obtained in EEC and EEU models in which symptoms are elicited in a carefully controlled environment with known allergen exposure (7, 8).

Mechanisms of Peptide-induced Tolerance

Modulation of Cellular Immunity

The immunological mechanisms of action of peptide immunotherapy were initially investigated in early clinical studies using prototype mixtures of peptides from Fel d 1 and also from the bee venom allergen Api m 1 (phospholipase A2). The investigators in these studies demonstrated changes in both cellular and humoral immunity, which have characteristics in common with existing immunotherapy approaches. However, the absence of conformational B cell epitopes on short peptides may explain the relatively weak humoral mechanistic signals seen in peptide immunotherapy compared with SCIT or SLIT employing whole allergen. In broad terms, several studies have provided evidence for a down-regulation of inflammatory T cell responses to allergen after therapy (12–18). These changes were also associated with a concomitant increase in immune regulatory signals such as the production of IL-10 in peripheral blood mononuclear cells (PBMCs) (14, 18). Changes in the pattern of cellular recruitment to sites of allergen challenge have also been observed.

Following peptide therapy, allergen challenge of the skin was demonstrated to lead to a significant increase in the number of Th1 cells ($CD4^+$/IFN-γ^+) and $CD25^+$ cells, suggesting that a combination of immune deviation (Th2 to Th1) and regulation (recruitment of regulatory T cells) may be important in controlling responses to allergen post-therapy (15). Alternatively, the accumulation of $CD25^+$ T cells observed in the study by Alexander and colleagues (15) may simply reflect the recruitment of activated T cells. In the same study, expression of transforming growth factor β (TGFβ) mRNA appeared to be increased in skin cells, whereas no increase in IL-10^+ cells was observed. In a related study, Smith and coworkers found no effect of peptide immunotherapy on the suppressive activity of $CD4^+CD25^+$ cells in peripheral blood (12), suggesting that naturally occurring regulatory T cells may not play a significant role in the mechanism of action. This observation is supported by the results of murine studies in which no increase in the absolute numbers of $Foxp3^+$ T cells was observed in the lungs of mice after successful peptide therapy (19). On the basis of an assessment of the intensity of Foxp3 staining (20), however, peptide immunotherapy may increase the amount of this regulatory transcription factor per cell, resulting in enhanced suppressive capability. To date, to the best of our knowledge, no functional suppression assays have been performed in the model in question to formally test this hypothesis.

Although the role of classical (i.e., natural) Foxp3$^+$ regulatory T cells in peptide immunotherapy remains incompletely defined, allergen-specific, IL-10–secreting, inducible regulatory T cells may be responsible for the increases in PBMC-derived (human) and lung digest–derived (murine) IL-10 observed in several studies. To assess the antigen-specific suppressive function of such cells, Verhoef and colleagues (17) mixed CD4$^+$ cells isolated from peripheral blood before and after peptide therapy with CD4$^-$ cells that had been labeled with a cell division tracking dye prior to culture with allergen. These researchers demonstrated that allergen-specific proliferative responses of memory T cells were reduced compared with baseline samples following therapy (17). These data support the hypothesis that peptide immunotherapy can induce a population of inducible, allergen-specific CD4$^+$ T cells. The dependence of *in vitro* suppression on IL-10 was not evaluated in this model; however, blockade of IL-10 receptors with a monoclonal antibody in a murine model demonstrated that suppression of multiple inflammatory outcomes was dependent on this cytokine (19).

The issue of whether peptide immunotherapy using limited numbers of peptides from selected allergens has the potential to modulate responses to complex mixtures of allergens (such as grass pollen or HDM allergens) has been addressed by studying the extent to which peptide immunotherapy might generate a tolerogenic environment in which T cells targeted by the therapy take on regulatory characteristics and then subsequently exert their regulatory activity on T cells specific for other epitopes of the allergen (intramolecular tolerance) or, indeed, other allergen molecules present in the immediate microenvironment (intermolecular tolerance). Evidence for intramolecular tolerance was obtained in a study where PBMCs were archived before and after peptide immunotherapy with a prototype mixture containing 12 peptides from Fel d 1. An additional four peptides not administered to the patients in that study were also available to test recall responses posttherapy (19). The results showed that, in addition to modulating the immune response to the treatment peptides themselves, peptide therapy also modified the response to nontreatment peptides from the same allergen, thereby providing an example of intramolecular tolerance in a clinical setting. More recently, my research group has obtained unpublished data demonstrating the upregulation of IL-10 production in response to allergen proteins not represented in the peptide therapy, thereby providing some evidence to support the induction of intermolecular tolerance. Additional support for these observations has been provided through *in vivo* experimental models (19, 20). Mice jointly sensitized to HDM allergens and ovalbumin (OVA) were treated with peptides from the HDM allergen Der p 1 (20). In addition to downregulating HDM responses, Der p 1 peptide therapy reduced inflammatory responses to OVA rechallenge, demonstrating the induction of intermolecular tolerance. Treatment was associated with increased IL-10 in lung homogenates and increased numbers of CD4$^+$IL-10$^+$ T cells (but not B cells) in the lung. We also measured levels of IL-35, a cytokine that has been associated with the induction of regulatory T cells (21, 22) and the loss of airway hyperresponsiveness in a murine model of immune tolerance induced with OVA and LPS (23). We saw no increase in IL-35 in this model, suggesting that, in peptide immunotherapy, IL-10 rather than IL-35 may be more closely associated with the induction of tolerance. Further experiments are required to confirm the validity of these observations.

Modulation of Humoral Immunity

SIT employing whole allergens is associated with the induction of allergen-specific IgG, most notably IgG4 and, in some reports, IgA. The potential role of these antibodies, which have been referred to as "blocking antibodies," remains unclear, although it is widely held that such antibodies contribute to clinical efficacy through neutralization of allergen before it is able to reach IgE molecules on the surface of effector cells, such as mast cells and basophils. In fact, no clear correlation exists between the efficacy of SIT and the levels of allergen-specific IgG induced during therapy. The lack of conformational B cell epitopes and the monomeric nature of linear epitopes within short peptides make the likelihood of stimulation of increased allergen-specific IgG production by peptide therapy low. Subsequent encounter with whole allergen in the environment, however, would introduce both T cell and B cell epitopes into an immune system that has been reprogrammed for regulation at the cellular level. Thus, B cell epitopes of the whole allergen could be recognized by B cell interaction with regulatory T cells secreting IL-10 and/or TGFβ, leading to the production of allergen-specific IgG4 or IgA. Evidence to support this hypothesis has been obtained in individuals with bee venom allergy who were treated with short peptide sequences. Five patients allergic to bee venom received incremental doses of three immunodominant T cell epitope peptides (24). A week after the last peptide injection, subjects were challenged subcutaneously with 10 μg of whole Api m 1. All five subjects tolerated the challenge without systemic sequelae. As expected, treatment with short peptides did not change allergen-specific serum IgE or IgG4 levels. Interestingly, however, subcutaneous challenge with whole allergen resulted in sharp increases in both allergen-specific IgE and IgG4, with the latter being substantially higher than the former.

Induction of allergen-specific antibodies was also observed in a second bee venom peptide immunotherapy study in which similar peptides were used to treat subjects with mild bee venom allergy (18). PBMC inflammatory responses to purified allergen and whole bee venom were significantly reduced following therapy, and IL-10 production increased. Allergen-specific IgG, IgG$_4$, and IgE levels were measured before, during, and after treatment. A modest (statistically significant) but transient increase in allergen-specific IgG and IgG4 was observed during and after treatment. The functional significance of this increase remains to be determined, as does the role of whole allergen challenge after therapy in the induction of antibody responses in the study by Tarzi and colleagues. In a model of peptide immunotherapy in which mice sensitized to both HDM allergens and OVA were treated with HDM peptides, a significant decrease in the numbers of CD19$^+$ B cells was observed in lung tissue digests (20). However, no decreases in allergen-specific IgE were observed in the sera of these animals. Because local IgE production in the respiratory mucosa has been demonstrated in samples from subjects with allergy (25–27), it is possible that reduced numbers of B cells may result in lower local concentrations of IgE.

Route of Administration and Modulation of Immunity

Traditional SIT is administered by subcutaneous injection, resulting in the formation of a depot of allergen in the subcutaneous fat and leading to slow dissemination of allergen into the systemic circulation. To date, the majority of clinical trials of peptide therapy demonstrating clinical efficacy have been performed with intradermal administration of peptide, which is thought to access the systemic circulation more rapidly and also to load local skin antigen-presenting cells (e.g., Langerhans cells), leading to presentation in local draining lymph nodes. The importance of Langerhans cells in the induction of immune tolerance to allergens has been highlighted in mechanistic studies of SLIT. Oral Langerhans cells constitutively express the high-affinity receptor for IgE (FcεRI) and Fcγ receptors, allowing them to bind allergen, and are the main dendritic cell subset in the oral mucosa (28). These cells display an immature phenotype. Upon Toll-like receptor 4 (TLR4) ligation, they up-regulate IL-10 and TGFβ production, resulting in their ability to induce IL-10 and TGFβ-producing T cells with regulatory characteristics (29, 30). The role of dermal Langerhans cells in peptide immunotherapy has not been studied to date. However, unpublished data obtained by my research group suggest that a substantial proportion of intradermally administered peptide remains in the skin bound to local antigen-presenting cells, including Langerhans cells.

To compare the intradermal and subcutaneous routes in an escalating dose cohort study (31), a seven-peptide mixture of Fel d 1 peptides (Cat-PAD) was administered in a single injection to subjects allergic to cats. The primary outcome was the surrogate clinical outcome measure of reduction in the magnitude of the cutaneous late-phase reaction following whole allergen challenge. Administration of peptides via the intradermal route was associated with a dose-dependent inhibition of the cutaneous late-phase response, whereas inhibition of this reaction was very heterogeneous and not dose-dependent following subcutaneous administration. Thus, on the basis of the results produced in this study by Worm and coworkers, albeit with small numbers of subjects and limited clinical outcomes, it appears that the intradermal route may be superior to the subcutaneous route in peptide immunotherapy (31).

Interestingly, the peptide-induced tolerance observed following intradermal administration was not observed following inhalation of peptides, despite the fact that both routes were equivalent in their ability to elicit a biological response in subjects with asthma (32). Although it has not been possible to measure systemic exposure following intradermal versus inhaled exposure routes, it is likely that insufficient peptide enters the systemic circulation following inhalation to allow modulation of peripheral allergen-specific T cells. Thus, T cell tolerance may occur as a result of systemic presentation to naïve or memory T cells in the relative absence of inflammatory and innate signals (e.g., TLR signaling or inflammatory cytokines).

In summary, peptide immunotherapy appears to share some, but not all, immunological mechanisms of action with whole allergen immunotherapy (SCIT and SLIT). Cellular responses appear to be broadly similar with regard to the induction of IL-10 and regulatory T cell populations. Humoral responses may differ, with only limited evidence supporting the induction of allergen-specific IgG in subjects treated with peptides. Further insight into the commonalities and differences between these related approaches may inform further optimization of allergen immunotherapy. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

References

1 Noon L. Prophylactic inoculation against hay fever. *Lancet* 1911;177: 1572–1573.

2 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;341:468–475.

3 Durham SR, Emminger W, Kapp A, de Monchy JG, Rak S, Scadding GK, Wurtzen PA, Andersen JS, Tholstrup B, Riis B, *et al*. SQ-standardized sublingual grass immunotherapy: confirmation of disease modification 2 years after 3 years of treatment in a randomized trial. *J Allergy Clin Immunol* 2012;129:717–725.e5.

4 Novak N, Bieber T, Allam JP. Immunological mechanisms of sublingual allergen-specific immunotherapy. *Allergy* 2011;66:733–739.

5 Kiel MA, Röder E, Gerth van Wijk R, Al MJ, Hop WC, Rutten-van Mölken MP. Real-life compliance and persistence among users of subcutaneous and sublingual allergen immunotherapy. *J Allergy Clin Immunol* 2013;132:353–360.e2.

6 Niespodziana K, Focke-Tejkl M, Linhart B, Civaj V, Blatt K, Valent P, van Hage M, Grönlund H, Valenta R. A hypoallergenic cat vaccine based on Fel d 1-derived peptides fused to hepatitis B PreS. *J Allergy Clin Immunol* 2011;127:1562–1570, e6.

7 Hafner RP, Couroux P, Armstrong K, Salapatek AM, Patel D, Larche M. Persistent treatment effect achieved at one year after four doses of Der p derived synthetic peptide immuno-regulatory epitopes in an exposure chamber model of house dust mite allergy. *J Allergy Clin Immunol* 2014;133:AB289. (Abstract).

8 Ellis AK, Frankish CW, Armstrong K, Larche M, Steacy L, Hafner RP. Treatment with synthetic peptide immuno-regulatory epitopes derived from grass allergens leads to a substantial reduction in grass allergy symptoms in the environmental exposure unit. *J Allergy Clin Immunol* 2014;133:AB290. (Abstract).

9 Worm M, Patel D, Creticos PS. Cat peptide antigen desensitisation for treating cat allergic rhinoconjunctivitis. *Expert Opin Investig Drugs* 2013;22:1347–1357.

10 Patel D, Couroux P, Hickey P, Salapatek AM, Laidler P, Larché M, Hafner RP. Fel d 1-derived peptide antigen desensitization shows a persistent treatment effect 1 year after the start of dosing: a randomized, placebo-controlled study. *J Allergy Clin Immunol* 2013;131:103–109.e7.

11 Nelson HS. Subcutaneous immunotherapy versus sublingual immunotherapy: which is more effective? *J Allergy Clin Immunol Pract* 2014;2:144–149, quiz 150–151.

12 Smith TR, Alexander C, Kay AB, Larché M, Robinson DS. Cat allergen peptide immunotherapy reduces $CD4^+$ T cell responses to cat allergen but does not alter suppression by $CD4^+CD25^+$ T cells: a double-blind placebo-controlled study. *Allergy* 2004;59: 1097–1101.

13 Oldfield WL, Kay AB, Larché 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;167:1734–1739.

14 Oldfield WL, Larché 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.
15 Alexander C, Ying S, B Kay A, Larché M. Fel d 1-derived T cell peptide therapy induces recruitment of $CD4^+CD25^+$; $CD4^+$ interferon-γ^+ T helper type 1 cells to sites of allergen-induced late-phase skin reactions in cat-allergic subjects. *Clin Exp Allergy* 2005;35: 52–58.
16 Alexander C, Tarzi M, Larché M, Kay AB. The effect of Fel d 1-derived T-cell peptides on upper and lower airway outcome measurements in cat-allergic subjects. *Allergy* 2005;60:1269–1274.
17 Verhoef A, Alexander C, Kay AB, Larché M. T cell epitope immunotherapy induces a CD4+ T cell population with regulatory activity. *PLoS Med* 2005;2:e78.
18 Tarzi M, Klunker S, Texier C, Verhoef A, Stapel SO, Akdis CA, Maillere B, Kay AB, Larché M. Induction of interleukin-10 and suppressor of cytokine signalling-3 gene expression following peptide immunotherapy. *Clin Exp Allergy* 2006;36:465–474.
19 Campbell JD, Buckland KF, McMillan SJ, Kearley J, Oldfield WL, Stern LJ, Grönlund H, van Hage M, Reynolds CJ, Boyton RJ, *et al*. Peptide immunotherapy in allergic asthma generates IL-10-dependent immunological tolerance associated with linked epitope suppression. *J Exp Med* 2009;206:1535–1547.
20 Moldaver DM, Bharhani MS, Wattie JN, Ellis R, Neighbour H, Lloyd CM, Inman MD, Larché M. Amelioration of ovalbumin-induced allergic airway disease following Der p 1 peptide immunotherapy is not associated with induction of IL-35. *Mucosal Immunol* 2014;7: 379–390.
21 Collison LW, Workman CJ, Kuo TT, Boyd K, Wang Y, Vignali KM, Cross R, Sehy D, Blumberg RS, Vignali DA. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature* 2007;450: 566–569.
22 Collison LW, Pillai MR, Chaturvedi V, Vignali DA. Regulatory T cell suppression is potentiated by target T cells in a cell contact, IL-35- and IL-10-dependent manner. *J Immunol* 2009;182:6121–6128.
23 Whitehead GS, Wilson RH, Nakano K, Burch LH, Nakano H, Cook DN. IL-35 production by inducible costimulator (ICOS)-positive regulatory T cells reverses established IL-17-dependent allergic airways disease. *J Allergy Clin Immunol* 2012;129:207–215, e1–e5.
24 Müller 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;101:747–754.
25 Coker HA, Durham SR, Gould HJ. Local somatic hypermutation and class switch recombination in the nasal mucosa of allergic rhinitis patients. *J Immunol* 2003;171:5602–5610.
26 Takhar P, Corrigan CJ, Smurthwaite L, O'Connor BJ, Durham SR, Lee TH, Gould HJ. Class switch recombination to IgE in the bronchial mucosa of atopic and nonatopic patients with asthma. *J Allergy Clin Immunol* 2007;119:213–218.
27 Smurthwaite L, Walker SN, Wilson DR, Birch DS, Merrett TG, Durham SR, Gould HJ. Persistent IgE synthesis in the nasal mucosa of hay fever patients. *Eur J Immunol* 2001;31:3422–3431.
28 Allam JP, Niederhagen B, Bücheler M, Appel T, Betten H, Bieber T, Bergé S, Novak N. Comparative analysis of nasal and oral mucosa dendritic cells. *Allergy* 2006;61:166–172.
29 Allam JP, Peng WM, Appel T, Wenghoefer M, Niederhagen B, Bieber T, Bergé S, Novak N. Toll-like receptor 4 ligation enforces tolerogenic properties of oral mucosal Langerhans cells. *J Allergy Clin Immunol* 2008;121:368–374.e1.
30 Allam JP, Würtzen PA, Reinartz M, Winter J, Vrtala S, Chen KW, Valenta R, Wenghoefer M, Appel T, Gros E, *et al*. Phl p 5 resorption in human oral mucosa leads to dose-dependent and time-dependent allergen binding by oral mucosal Langerhans cells, attenuates their maturation, and enhances their migratory and TGF-β1 and IL-10-producing properties. *J Allergy Clin Immunol* 2010;126:638–645, e1.
31 Worm M, Lee HH, Kleine-Tebbe J, Hafner RP, Laidler P, Healey D, Buhot C, Verhoef A, Maillère B, Kay AB, *et al*. Development and preliminary clinical evaluation of a peptide immunotherapy vaccine for cat allergy. *J Allergy Clin Immunol* 2011;127:89–97, e1–e14.
32 Ali FR, Oldfield WL, Higashi N, Larché M, Kay AB. Late asthmatic reactions induced by inhalation of allergen-derived T cell peptides. *Am J Respir Crit Care Med* 2004;169:20–26.