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Allergen immunotherapy with cat allergen peptides

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Abstract Desensitising therapy for allergic diseases has changed little over almost a century of practice. Administration of increasing doses of extracts of allergen source material has been shown to be reproducibly effective when patients are carefully selected and appropriate concentrations of allergen employed. However, specific immunotherapy is limited by the interaction of specific IgE with allergen, leading to a relatively high frequency of adverse events including anaphylaxis and death. Several strategies have been developed to tackle this issue. Most of these rely on reducing the allergenicity of the treatment, whilst maintaining the immunogenicity. The use of short, synthetic peptide sequences corresponding to T-cell epitopes from the allergen has been shown to modify surrogate markers of allergy including cutaneous responses to allergen challenge and ex vivo parameters of T-cell activation. This review discusses recent advances in our understanding of the mechanisms and potential efficacy of this form of therapy.

Keywords Epitope · Asthma · Tolerance · T cell · Allergy

Introduction

The immune response to allergens, as in the case of most glycoproteins, consists of both humoral and cell-mediated elements. Since for many allergens (aeroallergens in particular) exposure (depending on season and geography) is ubiquitous, a large proportion of the population exhibit measurable immune responses to common allergens. The nature of the immune response to allergen determines the presence or absence of a clinical phenotype. Allergic individuals mount Th2 responses to the sensitising allergen that are characterised by the production of Th2 cytokines (IL-4, IL-5, IL-9, IL-11, IL-13) by specific T cells and the production of allergen-specific IgE by B cells [1, 2]. Whilst some non-allergic individuals may not generate measurable responses to certain allergens (particularly some house

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dust mite proteins), in general the response of non-allergic individuals has been characterised as a Th1 response. However, it is likely that this is an over simplification, not least because allergen challenge of non-allergic subjects (with notable exceptions) does not lead to a classical delayed-type hypersensitivity reaction. Recent data have implied that in health, immunity to allergens may be controlled by regulatory cells such as T cells that maintain homeostasis between pro-inflammatory and anti-inflammatory responses.

Allergic asthma is a disease displaying elements of both antibody-mediated and cell-mediated responses. The pathogenesis of asthma remains poorly understood. Models of provoked asthma in controlled clinical settings have provided useful information. For example, the respective roles of IgE, mast cells and basophils in the early asthmatic response are relatively well elucidated. The mechanisms underlying the late asthmatic reaction, on the other hand, are less well defined but are likely to involve multiple cell types and mediators. The influences of early mediator release and cell recruitment on the late-phase provide an extra layer of complexity. Animal models have limited value in understanding pathogenesis since they may not precisely reflect the disease in man. Airway tissue sampled either at baseline or after allergen challenge indicates that allergic asthma is a chronic inflammatory condition with increases in granulocytes (particularly eosinophils) and Th2 lymphocytes, and in the concentration of associated cytokines and chemokines [1, 2].

Allergen immunotherapy

For the majority of individuals allergic sensitisation results in relatively mild clinical disease that can be managed adequately with conventional palliative pharmacotherapy. However, more serious manifestations of allergic sensitisation such as asthma and anaphylaxis represent serious clinical phenotypes for which there is a significant unmet need with respect to curative treatment.

Disease-modifying therapy has been available for many decades in the form of conventional whole-allergen immunotherapy, often referred to as SIT (specific immunotherapy). First described at the beginning of the last century [3] SIT involves administration of increasing doses of whole-allergen extract, initially by injection but more recently by the sub-lingual route. The approach is widely used but has some significant drawbacks. The principal problems associated with this form of therapy are (1) the risk of systemic IgE-mediated reactions resulting from cross-linking by intact allergen, (2) the relatively crude and unstandardised extracts employed and (3) the length of time that effective desensitisation takes. For example, subcutaneous or intramuscular injections are given at least monthly for 3 or more years.

Approaches to reduce the incidence of IgE-mediated adverse reaction during immunotherapy have focused on reducing the interaction between therapeutic allergen preparation and specific IgE. Approaches have included the disruption of the tertiary structure of the protein with cross-linking agents such as glutaraldehyde [4], the introduction of point mutations or deletions that reduce the number of conformational B cell epitopes displayed by an allergen [5] and the delivery of allergen coupled to synthetic bacterial DNA sequences that sterically hinder interaction between allergen and IgE and drive T-cell responses towards a Th1 phenotype [6, 7]. During the past decade synthetic peptides derived from the amino acid sequence of the allergen have been produced and used to treat individuals allergically sensitised to common allergens. Most data have been obtained from peptides de-

rived from the major cat allergen Feld1 [8, 9, 10, 11, 12, 13], although more recently a number of studies have been performed with peptides from the bee venom allergen phospholipase A₂ [14, 15]. Initial studies with peptide immunotherapy identified a frequent, delayed response to peptides during which treated individuals reported allergic symptoms, including chest tightness, shortness of breath and wheeze several hours after peptide administration. We hypothesised that the late symptoms of allergy and chest tightness observed in some patients may have been due to the direct activation of allergen-specific T cells by the peptides leading to an IgE-independent component of bronchoconstriction that may contribute to overall asthma symptoms in everyday life.

In vivo peptide challenge

In order to reproduce peptide-induced late allergic symptoms a modified allergen challenge system was established. Asthmatic individuals allergic to cats were challenged with short peptides representing T-cell epitopes derived from the sequence of the major cat allergen Feld1. The peptides were large enough to act as T-cell epitopes but likely to be too short to cross-link neighbouring IgE molecules on the surface of mast cells and basophils [16].

Peptides of 16 or 17 amino acids in length were chemically synthesised. In vitro epitope mapping studies were performed in atopic and non-atopic individuals both with and without asthma in order to determine the regions of the molecule that induced the greatest T-cell activation. Initially three peptides, termed Feld chain 1 peptides (FC1P) were selected from chain 1 of the Feld1 molecule. The peptides were evaluated together with whole cat dander allergen for their ability to induce histamine release from an enriched population of basophils isolated from peripheral blood of sensitised cat allergic subjects. In contrast to whole-allergen extract, increasing concentrations of a mixture of the three peptides (FC1P) induced no significant histamine release. The data confirmed that the peptide preparation did not cross-link adjacent IgE molecules in the individuals examined. Based on these findings it was hypothesised that FC1P, when administered in vivo, would not induce an early-phase reaction.

Induction of peptide-mediated MHC-restricted isolated late asthmatic reactions

Four small cohorts of cat allergic asthmatic volunteers were administered single increasing doses of FC1P. A dose of 80 µg of each of the three peptides was required to induce isolated late asthmatic reactions (LARs) in a proportion (25%) of subjects. The magnitude of the response varied among those who developed such reactions with certain subjects experiencing a reduction in forced expiratory volume in 1 s (FEV₁) of as much as 65%. All subjects receiving peptide injection were monitored in the clinic for a minimum of 6 h after injection. Reversal of airway narrowing was readily achieved in all individuals experiencing LAR with the use of short acting β_2 agonists [16].

Subsequently, 40 cat allergic asthmatic subjects with mild to moderate disease, were challenged by intradermal injection of 80 µg FC1P. Nine of the 40 developed isolated LAR. The bronchial response to the peptide mixture was believed to be mediated by CD4⁺ T cells that have been shown to be present in increased numbers in bronchial biopsy sam-

ples from asthmatic individuals. Evidence of MHC restriction was sought and tissue typing was performed on all individuals to determine HLA class II expression. The results revealed that four of the nine subjects developing LAR expressed a human leukocyte antigen (HLA) DR13 allele, three a DR1 allele and a further three a DR4 allele. Despite the small numbers of individuals analysed, these frequencies appeared to be higher than that expected in a random sample of the population.

In order to test the hypothesis that these three DR molecules were capable of restricting the T-cell response to one or more of the FC1P peptides, presentation experiments were performed *in vitro*. Individual peptides were incubated with murine fibroblasts transfected with various HLA-DR molecules. Peptide-pulsed transfectants were then cultured with cat dander-specific T-cell lines that had been raised prior to exposure to peptides, from subjects experiencing isolated LAR. Two of the three peptides were shown to be restricted by DR1, 4 or 13. Based upon the MHC restriction observed in these experiments it was concluded that isolated LAR occurred as a direct result of T-cell activation in an MHC-restricted fashion, following intradermal administration of allergen-derived peptide sequences. However, the connection between T-cell activation and smooth muscle contraction remains to be firmly established.

The target organ specificity of the peptide-induced LAR (frequently associated with nasal congestion in these asthmatic-rhinitic subjects) suggested that the systemic delivery of peptides via the intradermal route may result in peptide-MHC interaction throughout the body, but that the physical location of memory effector T cells was likely to determine the clinical outcome of the challenge. The largest accumulation of allergen-specific T cells was likely to be at the site of both the disease and the site of the highest exposure to allergen.

The induction of antigen-specific T-cell hyporesponsiveness

In order to determine the reproducibility of bronchial reactions, individuals experiencing LAR following FC1P challenge were rechallenged with the same dose and via the same route at a variety of time intervals. A second challenge within a few weeks was associated with a marked attenuation of the LAR implying the induction of peptide-specific hyporesponsiveness or “tolerance”. In contrast, rechallenge after approximately 1 year or more was associated with the induction of LAR similar to the initial challenge. These results suggested that peptide administration induced a transient T-cell activation leading to an MHC-restricted LAR. After a period of a few days responsiveness to the peptide was markedly reduced or absent [16]. Precedents for the induction of immunological tolerance in the T-cell compartment (in mice) following transient T-cell activation have been described [17, 18, 19].

An important question in relation to these observations, and one not addressed in other studies, is whether the same cells are initially activated (inducing LAR) and subsequently become “tolerant” (perhaps as a result of anergy, active suppression or activation-induced cell death). An alternative explanation could be that memory effector cells in the lung and nose are initially activated by peptide challenge, and that this somehow leads to smooth muscle constriction and a LAR. Subsequently, and as a separate immunological event, peptides are presented to naive and memory T cells in lymph nodes in a “tolerogenic” form (perhaps by immature dendritic cells). Interestingly, it was possible to induce anti-

gen-specific hyporesponsiveness with Feld1 peptides in the absence of LAR, perhaps suggesting that it is indeed possible to dissociate these two events.

Peptide immunotherapy

Allervax cat: IPC-1/IPC-2

Peptide-based immunotherapy has been evaluated in a number of studies in recent years. The majority of studies have focused on individuals with allergic disease induced by cats. Norman and colleagues [8] administered four injections of peptide or placebo to 95 cat sensitive patients in three dose groups (7.5 µg, 75 µg and 750 µg per injection). Statistically significant changes in symptom scores were observed in the high-dose group. Both nasal and lung symptom scores improved, 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 demonstrated reduced IL-4 production in IPC-1/IPC-2 specific T-cell lines following therapy [20]. A similar reduction in IL-4 production was reported by Pène and colleagues [9] who also observed a reduction in allergen PD₂₀ in both high- and medium-dose regimens when compared to baseline, but not when compared to placebo.

Simons and colleagues [10] performed a double-blind, parallel group study administering weekly injections for 4 weeks of 250 µg of the same IPC-1 and IPC-2 preparation to 42 (21 active and 21 placebo) individuals with cat-induced rhinitis and/or asthma. Adverse events observed included late-onset symptoms of rhinitis, asthma and pruritis following treatment. Cytokine secretion profiles of peripheral blood mononuclear cells (PBMC) remained unaltered after therapy. No change in early- and late-phase skin responses to whole allergen was recorded at several time points (2, 6 and 24 weeks) after treatment.

In a multi-centre study of 133 subjects receiving eight injections of 750 µg IPC-1/IPC-2, Maguire and colleagues [11] reported an improvement in pulmonary function but only in those who had diminished baseline FEV₁. Moreover, improvements were evident at only a single time point (3 weeks) post therapy. Injections were given in two blocks of four, with two injections per week resulting in a total dose of 6 mg of each peptide. A large number of adverse events were reported in this study including some that required the use of epinephrine. In keeping with other studies evaluating IPC-1/IPC-2, late-onset adverse reactions diminished with successive doses of peptide, suggesting the induction of hyporesponsiveness.

Thus treatment of cat allergic subjects with IPC-1/IPC-2 resulted in some improvement in clinical and surrogate outcome measures but was associated with a relatively high frequency of adverse events. Early events were likely to be due to cross-linking of IgE by the peptides. By virtue of their relatively large monomeric size or their capacity to form homo- and heterodimers through cysteine residues they may have been able to cross-link adjacent IgE molecules on the surface of mast cells and/or basophils. Other, more common adverse events were characterised by symptoms of chest tightness and wheeze with an onset several hours after peptide administration. Based on the descriptions and kinetics of such reactions it is likely that these events were T-cell-dependent, IgE-independent, isolated LAR.

Multiple short overlapping peptides from Feld1

Intradermal injection of peptides from Feld1 resulted in isolated LAR followed by extended periods of hyporesponsiveness to re-challenge, suggesting the induction of a form of “immunological tolerance” [12, 16]. Using the cutaneous response to allergen challenge as a surrogate marker of allergic disease, the effect of peptide administration 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 Feld1 molecule in order to enhance the chances of providing T-cell epitopes capable of binding to a broad array of MHC alleles and thus covering the majority of the population. Areas of sequence displaying poor solubility due to the presence of large numbers of hydrophobic residues were excluded from the mixture. The results demonstrated that the magnitude of the cutaneous late-phase response (LPR) to allergen could be markedly reduced even after a single administration of peptide. Furthermore, several subjects who had received peptide but had not experienced a LAR also displayed equivalent reductions in the size of the cutaneous late response to whole allergen, suggesting that LAR are not required to achieve T-cell hyporesponsiveness to whole allergen.

Double-blind, placebo-controlled study

Based on the ability of a single dose of allergen-derived peptides to reduce the magnitude of the cutaneous LPR following allergen challenge, a double-blind, placebo-controlled clinical trial was initiated in which multiple incremental doses of MOP were administered intradermally without adjuvant [13]. 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 (Global Evaluation to Cat Exposure). Subjects were treated with a total of 90 µg of each of 12 peptides (16–17 amino acids in length mixed together in saline). Doses were administered at 3- to 4-day intervals. As the subjects were asthmatic, it was expected that approximately 50% of those on active treatment would develop peptide-induced, isolated LARs (characterised by a 20% or greater fall in FEV₁) during peptide up-dosing (based on previous observations). Twice as many subjects were recruited into the treatment arm of the study in order to test the hypothesis that the induction of isolated LAR was not a prerequisite for the induction of hyporesponsiveness. When LARs were induced, 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. Up-dosing was then reinstated with adjustments made in order to deliver the same cumulative dose to all subjects.

Two outcome assessments were performed after treatment at which baseline parameters were repeated. The first follow-up was 4–8 weeks after therapy and the second 3–9 months after treatment. 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 in the placebo group also experienced a reduction in FEV₁ of greater than 20%, indicating the relatively severe nature of the disease in the group as a whole.

Peptide treatment was associated with significant reductions in the magnitude of both early- and late-phase cutaneous reactions to intradermal challenge with the purified allergen Feld1 when compared to placebo. Reactions to whole cat dander allergen extract were

also significantly lower than baseline in treated patients. Reduction in early-phase reaction size was not evident at the first (4–8 week) follow-up, implying dissociation between the modulation of the IgE-mediated early-phase response 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. Thus it may be that T-cell responses are modified rapidly through, for example, reduction in IL-4 and IL-13 secretion or the induction of regulatory T cells.

Analysis of allergen-specific PBMC responses *in vitro* revealed marked reductions in proliferative and pro-inflammatory cytokines (IL-4, IL-13, IFN γ) at the initial outcome visit. However, levels of IL-4, IL-13 and IFN γ showed a less marked reduction from baseline at the second outcome visit. In contrast, IL-10 production was elevated at both follow-up time points. Reduction in pro-inflammatory cytokines together with a concomitant rise in IL-10 suggests the induction of active immunoregulation following peptide treatment. Although regulatory T cells have been characterised as producers of relatively high concentrations of IL-10, preliminary data suggest that the major source of IL-10 in PBMC following peptide treatment is monocytes.

Inhaled delivery of peptides

Feld1-derived T-cell peptides injected intradermally induced LARs followed by bronchial hyporesponsiveness to peptide, inhibition of the whole-allergen-induced late phase skin reaction and altered T-cell function *in vitro*. We hypothesised that similar changes would occur if peptides were delivered by the inhaled route. Peptides were nebulised in a small volume of saline for inhaled delivery. Peptides induced isolated LARs in a proportion of cat allergic asthmatics by inhalation with kinetics that were indistinguishable from the intradermal route and at doses very similar to those given intradermally [24].

In contrast to the intradermal route, repeat inhalation of peptides was not associated with abrogation of the LAR. In addition, inhaled peptides did not inhibit the cutaneous LPR to whole cat dander. Thus, unlike the intradermal route, LARs provoked by inhalation did not induce whole-allergen or peptide-specific hyporesponsiveness. This observation suggests that the route of administration may be critical in “tolerance” induced by T-cell peptides. However, administration of peptides via the nose may result in a qualitatively different immune response, leading to tolerance as demonstrated in animal models. Alternatively, the success of the intranasal route in animals may in reality be associated primarily with the high doses of peptide employed. Thus it may be that the critical factor in this form of immunological tolerance may be achieving a sufficiently high plasma concentration of peptides. Whilst this can be done via the inhaled route, much larger doses of peptide may be required—a strategy that may carry its own risks.

Mechanisms of peptide-induced tolerance

The ability of peptides to activate T cells and induce LARs appears to be unrelated to the ensuing immunomodulation observed following intradermal administration. Thus activation of memory T cells in the airways may result in bronchoconstriction whilst other mechanisms may be involved in induction of hyporesponsiveness. The fact that hyporesponsiveness has been observed in the absence of bronchoconstriction in certain subjects

supports this notion. Additionally, whilst activation of T cells manifest as a LAR can be observed within a few hours, hyporesponsiveness appears to take several days to become established.

Following intradermal administration, some peptides may enter the peripheral circulation via the cutaneous capillary bed, traffic to the lung and activate effector T cells. Additionally, some material may be taken up by dendritic cells in the periphery that then migrate to draining lymphoid tissue. The relatively low dose, solubility and inability to cross-link allergen-specific antibodies on the surface of antigen presenting cells may render the peptides tolerogenic in this form. Thus, peptides may load MHC class II molecules on the cell surface without providing an activation/maturation signal. Previous studies have demonstrated the presence of a significant percentage of “empty” MHC class II molecules on the surface of immature dendritic cells accompanied by active HLA-DM, a class II chaperone and peptide editing protein capable of loading exogenous peptides into empty MHC class II binding grooves [21, 22]. Thus, immature antigen-presenting cells recycling through lymphoid tissue may present allergen peptides to T cells without all of the relevant costimulatory signals associated with a proinflammatory response. In contrast, peptides delivered in small amounts by inhalation may be degraded rapidly and may not reach the lymphatics or peripheral circulation in sufficiently high concentrations to induce hyporesponsiveness.

Conclusions and future directions

Peptide challenge of allergic volunteers has provided new insight into the mechanisms underlying airway narrowing in the late asthmatic reaction. However, no direct link between the activation of peptide-specific T cells and bronchoconstriction has been established. Recent studies have suggested that Th2 cytokines in themselves may be sufficient to induce airway hyperreactivity [23], but whether this feature of asthma is truly representative of the response to allergen remains to be determined.

A prominent feature of whole-allergen immunotherapy is a relative increase in allergen-specific IgG (particularly IgG4). The latter may play a protective role by sequestering allergen before it can interact with IgE. Short synthetic peptides represent a potentially safer approach to immunotherapy for allergic diseases since they lack the ability to cross-link cell surface IgE on mast cells and basophils. However, this inability to cross-link cell surface Ig must also extend to B cells producing IgG antibodies specific for the allergen. This may result in a lack of IgG induction following peptide immunotherapy, and this may compromise efficacy. However, natural exposure to allergen following peptide-induced T-cell modulation may be sufficient to stimulate B cell responses to allergen in a non-Th2 cytokine milieu. The observation that peptide therapy in cat allergic asthmatics leads to a significant reduction in the magnitude of wheal and flare reactions may support this concept.

Finally, a number of murine studies have indicated that it may be possible to tolerise to multiple allergens following the administration of tolerogenic peptides from a single allergen. This concept holds great promise for the development of human therapies in allergy and other T-cell-mediated diseases. Studies of this kind in humans are eagerly anticipated.

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Reference

1. Kay AB (2001) Allergy and allergic diseases. First of two parts. *N Engl J Med* 344:30–37
2. Kay AB (2001) Allergy and allergic diseases. Second of two parts. *N Engl J Med* 344:109–113
3. Noon L (1911) Prophylactic inoculation against hay fever. *Lancet* I:1572–1573
4. Marsh DG, Norman PS, Roebber M, et al (1981) Studies on allergoids from naturally occurring allergens. III. Preparation of ragweed pollen allergoids by aldehyde modification in two steps. *J Allergy Clin Immunol* 68:449–459
5. Ferreira F, Ebner C, Kramer B, et al (1998) Modulation of IgE reactivity of allergens by site-directed mutagenesis: potential use of hypoallergenic variants for immunotherapy. *FASEB J* 12:231–242
6. Creticos PS, Eiden JJ, Balcer S, et al (2000) Immunostimulatory oligonucleotides conjugated to Amb a 1: safety, skin test reactivity and basophil histamine release. *J Allergy Clin Immunol* 105:S70
7. Creticos PS, Balcer S, Schroeder JT, et al (2001) Initial immunotherapy trial to explore the safety, tolerability and immunogenicity of subcutaneous injections of an Amb a 1 immunostimulatory oligonucleotide conjugate (Aic) in ragweed allergic adults. *J Allergy Clin Immunol* 107:S216
8. Norman PS, Ohman JL, Long AA, et al (1996) Treatment of cat allergy with T-cell reactive peptides. *Am J Respir Crit Care Med* 154:1623–1628
9. Pene J, Desroches A, Paradis L, et al (1998) Immunotherapy with Feld1 peptides decreases IL-4 release by peripheral blood T cells of patients allergic to cats. *J Allergy Clin Immunol* 102:571–578
10. Simons FE, Imada M, Li Y, et al (1996) Feld1 peptides: effect on skin tests and cytokine synthesis in cat-allergic human subjects. *Int Immunol* 8:1937–1945
11. Maguire P, Nicodemus C, Robinson D, et al (1999) The safety and efficacy of allervax cat in cat allergic patients. *Clin Immunol* 93:222–231
12. Oldfield WL, Kay AB, Larche M (2001) Allergen-derived T cell peptide-induced late asthmatic reactions precede the induction of antigen-specific hyporesponsiveness in atopic allergic asthmatic subjects. *J Immunol* 167:1734–1739
13. Oldfield WL, Larche M, Kay AB (2002) Effect of T-cell peptides derived from Feld1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. *Lancet* 360:47–53
14. Muller U, Akdis CA, Fricker M, et al (1998) 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* 101:747–754
15. Fellrath JM, Kettner A, Dufour N, et al (2003) Allergen-specific T-cell tolerance induction with allergen-derived long synthetic peptides: results of a phase I trial. *J Allergy Clin Immunol* 111:854–861
16. Haselden BM, Kay AB, Larche M (1999) Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. *J Exp Med* 189:1885–1894
17. Vidard L, Colarusso LJ, Benacerraf B (1994) Specific T-cell tolerance may be preceded by a primary response. *Proc Natl Acad Sci U S A* 91:5627–5631
18. Webb S, Morris C, Sprent J (1990) Extrathymic tolerance of mature T cells: clonal elimination as a consequence of immunity. *Cell* 63:1249–1256
19. Hoyne GF, Askonas BA, Hetzel C, et al (1996) 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* 8:335–342
20. Marcotte GV, Braun CM, Norman PS, et al (1998) Effects of peptide therapy on ex vivo T-cell responses. *J Allergy Clin Immunol* 101:506–513
21. Santambrogio L, Sato AK, Fischer FR, et al (1999) Abundant empty class II MHC molecules on the surface of immature dendritic cells. *Proc Natl Acad Sci U S A* 96:15050–15055
22. Santambrogio L, Sato AK, Carven GJ, et al (1999) Extracellular antigen processing and presentation by immature dendritic cells. *Proc Natl Acad Sci U S A* 96:15056–15061
23. Hakonarson H, Maskeri N, Carter C, et al (1999) Regulation of TH1- and TH2-type cytokine expression and action in atopic asthmatic sensitized airway smooth muscle. *J Clin Invest* 103:1077–1087
24. Ati TR, Oldfield WLG, Higashi N, et al (2003) Late asthmatic reactions induced by inhalation of allergen-derived T-cell peptides. *Am J Resp Crit Care Med* DOI: 10.1164/rccm.200305-690C