

Peptide modulation of allergen-specific immune responses

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In vitro peptide stimulation of allergen-reactive T-helper type 1 and type 0 cells, in the absence of costimulatory signals, induces anergy that is accompanied by the modulation of cell surface phenotype and changes in cytokine production. In experimental animal models, the administration of allergen-derived peptides may result in the downregulation of cytokine and antibody production, which is preceded by transient activation of CD4⁺ T cells, without the induction of effector immunity. Preliminary results of clinical trials using allergen-derived peptides for desensitization are becoming available and should provide some insight into the efficacy of peptide therapy in man.

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Introduction

The prevalence of allergic disorders is increasing, with up to 20% of the population of developed countries affected. In individuals who are genetically susceptible to atopic allergy, exposure to allergens induces the overproduction of T-helper type 2 (Th2) cytokines (IL-4, IL-5, IL-10 and IL-13) and the production of IgE antibodies [1–3]. This response is accompanied by the infiltration of inflammatory cells, such as mast cells, basophils and eosinophils, which release histamine, leukotrienes and other inflammatory mediators. In contrast, Th1-derived cytokines (IL-2 and interferon [IFN]- γ) dominate the response to allergens in non-atopic individuals [4]. Effective treatment for some allergic disorders can be achieved by immunotherapy with intact native allergens [5, 6–7]. However, in order to avoid the potential risk of anaphylaxis, which may occur when native allergens are administered, interest has focused on the development of immunotherapy using allergen-derived T-cell epitopes in the form of peptides (PS Norman, JL Ohman, abstract in *J Allergy Clin Immunol* 1994, 93:231; see also [8]). The problem remains as to the nature of qualitative and quantitative changes in immune responses that are induced by immunotherapy and what is required to mediate protection. From recent studies, however, it appears that allergen desensitization may be accompanied by the induction of Th1-derived cytokines [6, 7, 9].

The aims of this review are to assess two aspects of peptide-mediated regulation of allergen-specific T-cell responses and their potential importance in immunotherapy. Firstly, the immunological basis underlying the

inhibition of responsiveness and, secondly, the ability of peptides to selectively promote the production of Th1-type cytokines by Th0 cells, which are a major component of the T-cell repertoire in atopic individuals.

In vitro peptide-mediated anergy in allergen-specific CD4⁺ T cells

The ability of peptides to modulate the effector functions of human T cells *in vitro* was initially observed for human Th0 cell clones reactive for influenza virus haemagglutinin. It was reported that after exposure to suprainmunogenic concentrations of specific peptide (in the absence of antigen-presenting cells [APCs]), the T cells failed to proliferate to antigenic rechallenge, although they remained responsive to exogenous IL-2 [10]. Using the same experimental approach, it has now been observed that Th2 cells reactive for Der p 1, a major allergen derived from house dust mite (HDM), become anergic after stimulation with high doses of specific peptide [11]. Although they express CD40 ligand, these T cells fail to provide B-cell help for IgE synthesis even in the presence of exogenous IL-4 and IL-13 [12]. Thus, stimulation of Th2 cells with the peptide under defined conditions not only prevents clonal expansion of T cells, but also their ability to provide B-cell help.

Peptide-mediated anergy is associated with compound changes in the T-cell phenotype characterized by the downregulation of the TCR-CD3 receptor complex and enhanced expression of CD2 and CD25 [13, 14].

Abbreviations

APC—antigen-presenting cell; [Ca²⁺]_i—intracellular calcium ion; HDM—house dust mite; IFN—interferon; IL—interleukin; PLA₂—phospholipase A₂; TCR—T-cell receptor; Th—T helper.

The kinetics of the recovery of TCR-CD3 expression are variable for individual T-cell clones, as is the ability of peptide stimulation to comodule other cell surface proteins, such as CD4, CD11a/18 and CD44 [12]. As costimulation of B7-2 (CD86), but not B7-1 (CD80), provides an initial signal that induces naive T cells to produce IL-4 [15], it is appropriate to investigate into the phenotypic expression of the B7 family of ligands and their counter receptors CD28/CTLA-4 on anergic T cells. However, no detectable changes in the level of expression of cell surface CD80 or CD86 were observed on Th0 or Th2 cells that had become unresponsive after peptide modulation [12]. Furthermore, in the absence of APCs, stimulation with peptide induces anergy in allergen-specific human Th2 cells, irrespective of cell-surface expression of CD80 and CD86. Membrane expression of the counter receptor CD28 on anergic T cells remains unchanged or is downregulated, however, in contrast to the enhanced levels of this receptor that are found on activated T cells [12,13,16].

Receptor modulation alone does not account for the loss of function, and analysis of cytokine production has established that anergy in both Th1 and Th0 cells is characterized by a defect in IL-2 secretion [11,17-19]. Elevated levels of cytokine-specific mRNA, although not always accompanied by an increase in protein secretion as a result of post-transcriptional regulation, suggests that in human CD4⁺ T cells, activation precedes the induction of anergy [20]. When restimulated with their natural ligand, the anergic human Th0/Th2 cells fail to secrete IL-2, IL-4, IL-5 and IL-13, whereas these cytokines are produced on restimulation with mitogen [12]. Furthermore, the production of IFN- γ by some human allergen-specific Th0/Th2 cells is resistant to anergy induced by engagement of the TCR without costimulation [11]. Analysis of the cytokine production by anergic murine Th2 and Th0 clones indicates that they retain the ability to produce IL-4 on restimulation [21,22,23*]. This observation is relevant because in atopic individuals, a marked component of the allergen-specific T-cell repertoire consists of Th0 cells. Although unable to proliferate and provide B-cell help, anergic human T cells remain responsive to exogenous IL-2 and pretreatment with IL-2 can prevent the induction of anergy [24]. Furthermore, anergic T cells also proliferate to IL-4 and IL-15, but not exogenous IL-7, even though the receptors for these cytokines share a common γ chain (γ_c) (H Yssel, J Lamb, unpublished data). However, preincubation with IL-2, IL-4, IL-7, or cross-linking γ_c with antibody, prevents CTLA4-Ig induced unresponsiveness in alloreactive T-cell clones [25].

Restoration of TCR expression without recovery of function suggests that differential signalling through the TCR, and not receptor downregulation, accounts for the altered responsiveness in allergen-reactive T cells that have become anergic following peptide stimulation. Information about the intracellular signalling pathways that are involved in anergy in Th2 cells is limited

compared with that available for Th1 cells; this is, in part, because of the increased resistance of Th2 cells to the induction of unresponsiveness. Anergic human Th1 and Th2 cells expressing normal levels of TCR are defective in their ability to release intracellular calcium [Ca^{2+}]_i on restimulation [12,26]. In contrast, it has been observed that basal levels of [Ca^{2+}]_i are elevated in anergic murine Th1 cells, which also display increased phosphorylation of phospholipase C- γ 1 [21]. Furthermore, anergy induced in murine Th1 cells by an altered peptide ligand is associated with a unique pattern of TCR ζ chain phosphorylation and its failure to associate with Zap70 protein tyrosine kinase [27*].

TCR occupancy by peptide-MHC class II complexes without costimulation, can downregulate proliferation and B-cell help and can modulate cytokine production through the activation of selective intracellular signalling pathways. The practical application of these findings is to the development of immunotherapy.

***In vivo* experimental models of peptide modulation of allergen-specific responses**

The application of peptides to specific immunotherapy for the treatment of allergic diseases is only now beginning to be evaluated clinically. However, from the analysis of two independent murine models, information has been acquired on the ability of peptides to modulate the response of allergen-specific T cells *in vivo* [28,29*].

The first of these experimental systems examined the effects of peptides given by subcutaneous injection (the traditional route of administration in allergen desensitization) on responses to Fel d 1 [28]. Fel d 1 is the major allergenic protein produced by cats and consists of two disulfide-linked polypeptide chains [30,31], both of which are targets of T-cell recognition. Subcutaneous administration of two peptides (27 amino acids in length) derived from chain 1, induced peripheral T-cell tolerance to the whole allergen and, when restimulated *in vitro* with allergen-specific CD4⁺ T cells from peptide-treated mice, failed to secrete IL-2, IL-4 and IFN- γ . In addition, peptide treatment downregulated the *in vitro* production of antigen-specific IgE antibody [28]. A recombinant fusion protein containing multiple linked T-cell epitopes from chain 1 of Fel d 1, can also induce peripheral T-cell tolerance in mice when delivered subcutaneously [32*]. However, the mechanisms underlying the development of T cell non-responsiveness following subcutaneous injection of peptides or recombinant polypeptides have not yet been delineated.

Delivering soluble antigens through mucosal surfaces is an effective way of modulating the function of both antigen-specific CD4⁺ and CD8⁺ T cells [33-36] and has prompted investigation into the effects of these routes of administration on the regulation of T-cell

responses to the HDM allergens in mice [29•,37]. The intranasal administration of a single peptide containing the immunodominant T-cell epitope of Der p 1 to H-2^b mice can inhibit T-cell responses to the whole protein [29•]. The development of tolerance is long lasting (G Hoyne, J Lamb, unpublished data) and peptide treatment can successfully inhibit established antigen-specific T-cell responses [29•]. Treating mice with peptide intranasally induces a transient activation of CD4⁺ T cells that eventually leads to a selective downregulation of cytokine secretion, which would be consistent with the development of clonal anergy. Feeding large amounts of a recombinant fusion protein containing the immunodominant T-cell epitope of Der p 1 to H-2^b mice can, likewise, abrogate T-cell responses to the whole protein [37]. In both situations, prior treatment of mice with peptides results in a loss of antigen-dependent IL-2 secretion and cellular proliferation. Importantly, treatment with one peptide, either orally or intranasally, could inhibit T-cell responses to the whole protein. A similar finding is also obtained with subcutaneous injection of Fel d 1 peptides [28,32•]. The results of these two experimental models suggest, theoretically, that successful immunotherapy in patients may be achieved using a restricted number of immunodominant peptides. It remains to be established, however, whether chronic restimulation with allergen after peptide-mediated immunotherapy will lead to the induction of disease-associated T cells reactive to different epitopes [38,39].

In many instances, allergen-specific immune responses are chronic, presumably arising as a result of continuous allergen exposure. However, we have recently observed that murine MHC class II associated invariant chain peptide (CLIP) is homologous to a major H-2b-restricted T-cell epitope in Der p 1 (residues 15–29) and mice primed with Der p 1 develop T-cell responses that are cross-reactive to the sequence 15–29 and CLIP (N Kristensen, J Lamb, unpublished data). This cross-reactive response on a self peptide may provide an additional means of maintaining memory and perpetuating the response *in vivo* to allergens derived from HDM. Nevertheless, responses to the intact protein can be inhibited by intranasal administration of the peptide 15–29.

Clinical studies of peptide vaccination

Allergen immunotherapy has been practised for many decades, but changes in the immunological parameters that give rise to improvements in clinical symptoms in humans are ill-defined. In some instances, successful allergen desensitization correlates with a decrease in IL-4 secretion by antigen-specific CD4⁺ T cells, but appears to have little immediate effect on the levels of circulating allergen-specific IgE [7], yet, these patients showed improvement in their clinical symptoms. Considerable

progress has been made in the clinical use of oral tolerance in the treatment of autoimmune diseases in human patients [35], whereas oral tolerance with allergen extracts has met with only limited success clinically. Improvements in the efficacy of oral tolerance in allergen immunotherapy, however, might be achieved with the use of purified recombinant allergens.

On the basis of the results obtained from an experimental mouse model, a clinical trial was established to test the efficacy of synthetic peptides derived from chain 1 of Fel d 1 in the treatment of the clinical symptoms of cat allergic patients. Primary indications from phase I and II clinical trials are that patients have tolerated the injections quite well with limited side effects (PS Norman, JL Ohman, AA Long, abstract in *J Allergy Clin Immunol* 1994, 93:231). An independent trial used synthetic peptides derived from the major bee venom allergen phospholipase A₂ (PLA₂) to successfully desensitize two bee venom allergic patients [8•]. The patients were desensitized with an equimolar mixture of three immunogenic peptides that had previously been shown to stimulate the response of PLA₂-specific CD4⁺ T-cell clones [40]. The patients tolerated the peptide injections well with minimal side effects, and displayed a greater decrease in the levels of allergen-specific IgE and IgG to both intact bee venom and PLA₂ than patients who underwent desensitization with whole bee venom.

Conclusions

Information on peptide modulation of allergen-specific immune responses is expanding; nevertheless, a number of questions need to be answered before the transition from experimental systems to clinical immunotherapy can take place. These include assessment of the duration and stability of peripheral tolerance and the degree of plasticity in antigen reactivity of the Th2 repertoire after desensitization. The susceptibility of memory Th2 cells to tolerance induction is controversial and suggests that targeting naive T cells with a view to inducing allergen-specific Th1 responses may offer a viable approach to immunotherapy [41]. Finally, the long-term efficacy of immunotherapy is linked to the ability to generate and maintain memory either in a tolerant Th2 population or preprogrammed Th1 population. Although cross-reactivity between certain sequences of allergenic and self proteins may contribute to the chronicity of the response, it may be possible to exploit this aspect of T-cell recognition in maintaining a memory Th1 response.

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