

Review article

Investigating T cell activation and tolerance in vivo: peptide challenge in allergic asthmatics

Trevor R.F. Smith, Mark Larché*

*Department of Allergy and Clinical Immunology, Imperial College London, Faculty of Medicine, National Heart and Lung Institute,
Dovehouse Street, London SW3 6LY, United Kingdom*

Abstract

In the atopic allergic individual, challenge with allergen elicits manifestations of both the humoral (IgE-mediated or early-phase reaction) and the cell-mediated immune response (late-phase reaction). Detailed study of late-phase cell-mediated events is confounded by the effects of the earlier IgE-mediated response which results in mast cell and basophil activation. Thus the relative contributions to allergic inflammation of individual cell types, such as T cells and eosinophils, are difficult to define.

In this review we describe experiments, largely from our own group, in which we have attempted to dissociate early and late allergic reactions. To this end, cell-mediated responses were induced in the absence of preceding IgE-mediated events, by the delivery of synthetic peptides representing T cell epitopes of the allergen. Activation of T cells resulted in airway narrowing and an increase in airway reactivity to non-specific stimuli.

Furthermore, we describe the induction of antigen-specific hyporesponsiveness or “tolerance” following intradermal, but not mucosal, peptide delivery. The induction of peptide-induced hyporesponsiveness could be temporally dissociated from the initial T cell activation resulting in bronchoconstriction and likely occurred through a different mechanism.

Analysis of in vitro allergen responses of peripheral blood cells revealed that hyporesponsiveness was associated with reductions in both Th1 and Th2 cytokines, together with a concomitant increase in the regulatory cytokine IL-10.

We conclude that activation of T cells in vivo may result in manifestations of chronic allergic inflammation including bronchoconstriction and hyperreactivity. Additionally, when administered systemically at low dose, peptides may induce long-lasting hyporesponsiveness in the T cell compartment, through a mechanism that is associated with induction of IL-10.

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1. Introduction

Unlike many other forms of immunopathology, allergic diseases can be readily studied in vivo in human subjects. This is the result of the relative ease with which objective measurements of immune activation can be obtained following challenge with the sensitising antigen (allergen). For example, allergen challenge in the skin

leads to an acute, or early-phase reaction (EPR; weal and flare), within 15 min. The reaction is the result of allergen crosslinking of mast cell surface-bound, specific IgE. The magnitude of this reaction is easily measured and represents a surrogate marker of the activity of the humoral immune response. In an asthmatic individual, reductions in parameters of lung function (such as FEV₁; forced expiratory volume in 1 s) 15–30 min after inhaled allergen challenge, provide another manifestation of the antibody-mediated arm of the allergen-specific immune response.

Following measurement of the EPR, the cell-mediated component of the immune response to allergen can be

* Corresponding author. Tel.: +44 20 7351 8372; fax: +44 20 7376 3138.

E-mail address: m.larche@imperial.ac.uk (M. Larché).

measured in the late-phase reaction (LPR). This reaction may be measured through a number of parameters depending upon the target organ. The LPR peaks (in magnitude) at approximately 3–9 h after challenge and is generally resolved by 24 h. In the skin, the LPR is characterised by the presence of the four cardinal signs of inflammation; calor, dolor, rubor and tumor. In the lung, the LPR (late asthmatic reaction; LAR) is characterised by a reduction in FEV₁ (measurable by spirometry) first observed 2–3 h after challenge, peaking at 6–9 h and resolving by 24 h. Biopsy of the challenge sites (skin, nasal mucosa, bronchial mucosa) 6–24 h after challenge reveals infiltration of Th2 cells and eosinophils, accompanied by increased levels of Th2 cytokines such as IL-4, IL-13 and IL-5, together with T cell- and eosinophil-active chemokines.

Thus, allergic sensitisation to well defined antigens (the majority of sensitising allergens have been identified and their genes cloned and sequenced) provides a unique clinical model in which to evaluate immunological interventions in the safety of the clinical laboratory and within a single day.

We have exploited this model in a series of studies in which allergic subjects were challenged with T cell peptide epitopes identified (and then chemically synthesised) from their sensitising allergen. In particular we have evaluated the clinical and immunological effects of T cell epitopes from the major allergen of cat dander Fel d 1, in subjects with allergic asthma triggered by cat exposure. Additionally and more recently, studies have also been performed with T cell epitopes from the major bee venom allergen phospholipase A₂ (PLA₂) in venom allergic subjects.

We hypothesised that challenge with T cell peptide epitopes would result in stimulation of T cell responses in the absence of activation of the humoral arm of the response to the intact allergen molecule. Thus, IgE crosslinking by allergen and the ensuing mast cell and basophil activation would be avoided, enabling study of the contribution of T cells, in isolation, to allergic inflammation.

2. T cell peptide epitope challenge in cat allergic asthmatic subjects

Previous studies on the location of T cell epitopes within the Fel d 1 molecule identified the amino terminus of chain 1 of the heterodimeric (chain 1 and chain 2) molecule [1,2]. In our own studies, Fel d 1 chain 1 was evaluated in peripheral blood mononuclear cell (PBMC) proliferation and cytokine assays [3]. For the first peptide challenge studies, three peptides, termed FC1P (Fel d Chain 1 Peptides) were selected from chain 1. The peptides were evaluated together with whole cat dander allergen for induction of histamine release from an

enriched population of basophils (PBMC) isolated from peripheral blood of sensitised cat allergic subjects. No significant histamine release was detected to the peptide mixture [4], indicating that the three peptide mixtures had substantially less mast cell-activating capacity than whole allergen molecules, as expected.

Peptides were administered to cat allergic asthmatic subjects by intradermal injection into the volar aspect of the forearm. Physical changes at the site of injection were monitored together with lung function (FEV₁) for 6–8 h, in order to capture any clinical manifestations of challenge. For safety reasons, small groups of subjects were challenged at each dose prior to dose elevation. Following the dose ranging study it was established that 80 µg of each of the three peptides, in a mixture, was able to induce bronchoconstriction in a proportion (25%) of subjects [4]. The reduction in airway calibre (internal diameter) was first observed approximately 3 h after intradermal administration and peaked at 6 h. As a result of the kinetics and spirometric appearance of these events (very similar to the late-phase reaction observed following whole allergen challenge but not preceded by an EPR), they were termed isolated late asthmatic reactions (LARs). The magnitude of the response varied among individuals with certain subjects experiencing a reduction in FEV₁ of as much as 65%. Interestingly, since the reduction in airway calibre occurred gradually (unlike an IgE-mediated early reaction), subjects experiencing isolated LAR felt well, and reported the reaction as being similar to natural exposure to cats. Reversal of airway narrowing was readily achieved in all individuals experiencing LAR with the use of short acting β_2 agonists, implying a prominent role for smooth muscle constriction in these reactions.

3. MHC restriction of peptide-induced LAR

In the first major study, 40 cat allergic asthmatic subjects, with mild to moderate disease, were challenged intradermally with FC1P [4]. Nine of the 40 developed isolated LAR at the single dose employed. Since the response to the peptide mixture was believed to be T cell-mediated, evidence of MHC restriction was sought. Tissue typing was performed at HLA class II loci. Four of the nine subjects developing LAR were identified as expressing a human leukocyte antigen (HLA)-DR13 allele, three a DR1 allele and a further three a DR4 allele. Despite the small number of individuals analysed, these frequencies were higher than that expected in a random sample of the population. Subsequent studies revealed a dose–response effect in peptide challenge [5]. Thus, it may be that in this single dose study, potential

reactors were overlooked as a result of their insensitivity to the peptide dose employed.

In order to test the hypothesis that one or more of these three DR molecules was 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 allergen extract-specific T cell lines from subjects experiencing isolated LAR. One peptide was found to bind to DR1 (0101) and DR13 (1301 & 1302), another peptide bound DR4 (0405 & 0408). Based upon the MHC restriction observed in these experiments it was concluded that isolated LAR occurs as a direct result of T cell activation, in an MHC-restricted fashion, following intradermal administration of allergen-derived peptide sequences. Subsequent studies involving challenge with a larger number (12; termed MOP; multiple overlapping peptides) of peptides from both chains of the Fel d 1 molecule demonstrated that providing additional peptides capable of binding to a broader range of MHC molecules resulted in increased frequency of isolated LAR [5].

4. Target organ reactions: systemic versus local peptide delivery

The target organ specificity of peptide-induced LAR, despite the distal skin challenge, is noteworthy. Immune responses to inhaled aeroallergens are detectable in the blood of atopic and many non-atopic individuals. However, it may be expected that responses in the allergen-exposed respiratory mucosa and draining lymphatics would be more robust as a result of chronic challenge. Interestingly, many of the asthmatic subjects experiencing isolated LAR also reported nasal congestion, compatible with their allergic rhinitic status. These observations support the hypothesis that peptides injected at a distal skin site, enter the circulation rapidly, perhaps via the cutaneous capillary bed, and become systemically distributed. Subsequently, direct binding to MHC expressed on both professional and non-professional antigen presenting cells (APC) and presentation to specific T cells may result in bronchoconstriction and nasal congestion in organs harbouring substantial allergen-specific memory T cell pools.

Since the kinetics of T cell-dependent bronchoconstriction may have been determined, to some extent, by the distal intradermal challenge site, an inhalational challenge model was established. We hypothesised that isolated LAR would occur if peptides were delivered by the inhaled route and that the manifestation of bronchoconstriction would be temporally identical to

intradermal delivery. MOP induced isolated LARs in cat allergic asthmatics whether delivered by inhalation or intradermal injection [6]. Peptides were nebulised in a total volume of 1 ml of saline and inhaled by tidal breathing. A percentage of individuals developed isolated LAR at doses very similar to those given intradermally. In one subject, just 100 ng of peptide was sufficient to reduce FEV₁ by 20%. The time course of induction of LAR appeared identical, regardless of the route of administration. This observation supports the notion that peptides administered intradermally exert their effects by entering the blood stream rather than through the local draining lymphatics. LARs induced via the inhaled route were accompanied by modest but significant sputum eosinophilia and elevated concentrations of total sputum cysteinyl leukotrienes (LTC₄/D₄/E₄) [6]. However, more recent unpublished data generated through bronchoscopy suggest that recruitment of eosinophils into the lung is not a prominent feature of inhaled peptide-induced LAR, in agreement with an earlier bronchoscopic evaluation of LAR provoked by intradermal challenge [7].

5. The induction of T cell “tolerance” with peptides

In order to gather data concerning the reproducibility of peptide-induced LAR, subjects were rechallenged with the same dose, via the same route (intradermal) at a variety of time intervals from 3 days to 68 weeks [4,5]. Rechallenge within a few weeks was associated with a markedly reduced or absent LAR. This observation suggested 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 primary challenge.

These data 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. Similar observations have been made in various murine models [8–10].

The implication from previous studies was that transient T cell activation by antigen under certain conditions (for example presentation of antigen by non-professional APC) was followed by the induction of anergy or clonal deletion of the same population. However, an alternative explanation is that the two events are separate, the activation event occurring in the memory effector T cell compartment in the target organ (and perhaps associated lymphatics), and the “tolerance” event occurring systemically in the lymphatics and targeting central memory and naïve T cells. In the peptide challenge model we have demonstrated that it is possible to induce T cell hyporesponsiveness in the

absence of LAR, suggesting that it is indeed possible to dissociate these two events. Eight cat allergic asthmatic individuals received a mixture of overlapping peptides from Fel d 1 by intradermal injection. The dosing regimen was incremental (0.1, 1.0, 5, 10, 25 µg) with a 2 week dose interval. None of the eight subjects experienced a LAR (reduction in FEV₁ of 20% or greater), despite the fact that the cutaneous LPR following whole allergen extract challenge after therapy was markedly reduced and that non-specific bronchial hyperreactivity (measured by histamine challenge) was also significantly improved (Alexander et al., unpublished data).

Based on the ability of a single dose of allergen-derived peptides to reduce the magnitude of the cutaneous LPR following allergen challenge [5], a double-blind, placebo-controlled (DBPC) clinical trial was initiated in which multiple incremental doses of MOP were administered intradermally in saline [11]. Twenty-four cat allergic asthmatic subjects were treated with a total of 90 µg of each of 12 peptides (MOP). Doses were administered at 3–4 day intervals. In a previous study [5] a dose of 5 µg of peptide (the starting dose for the DBPC) resulted in 50% of subjects developing LAR. Thus, it was expected that approximately 50% of those on active treatment would develop peptide-induced LAR during peptide up-dosing. Therefore, 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. For safety reasons, 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 follow-up 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. A total of eight subjects (50%) 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 groups 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 Fel d 1 when compared to placebo. Reactions to whole cat dander allergen extract were also significantly reduced from 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 EPR and

the largely T cell-driven LPR which was reduced at the first (4–8 week) follow-up and further reduced at the later time point [11].

6. In vitro evaluation of immune modulation

In addition to measurements of clinical surrogates such as the cutaneous LPR following whole allergen challenge the effects of peptide administration on peripheral blood responses to allergen were also evaluated in vitro. Paired blood samples (before and after peptide) were collected from individuals receiving either single or multiple administrations of peptides. PBMC were cultured with whole allergen extract, with individual peptides from the challenge mixture and also with a small number of Fel d 1 peptides that were not included in the challenge material. Consistent results were obtained in several studies. Peptide challenge/therapy resulted in reduced allergen-specific proliferation in PBMC together with reductions in both Th2 (IL-4, IL-13) and Th1 (IFN-γ) cytokine production [5,11]. Interestingly, levels of IL-10 in culture supernatants were found to be significantly elevated after peptide therapy [11]. Recent studies in subjects treated with bee venom peptides have revealed the same pattern of immune modulation (M. Tarzi et al., unpublished data) [12,13].

7. Differential induction of hyporesponsiveness through the intradermal and inhaled routes

Fel d 1-derived T cell peptides injected intradermally, or inhaled, induced late asthmatic reactions (LARs). However, only intradermal challenge was followed by bronchial hyporesponsiveness to peptide, inhibition of the whole allergen-induced late phase skin reaction, and modulation of PBMC responses to allergen in vitro.

Repeated inhalation of peptides was not associated with abrogation of the LAR [6]. In addition, inhaled peptides did not inhibit the cutaneous late phase response (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. Inhaled (intranasal) delivery of peptides in several murine models has been shown to be associated with the induction of tolerance. Administration of peptides via the nose may result in a qualitatively different immune response. Alternatively, the success of the intranasal route in animals may in reality be associated primarily with the relatively high doses of peptide employed. Thus, a critical factor in the induction of immunological tolerance may be achieving

a sufficiently high plasma concentration of peptides. This can be done via the inhaled route but much larger doses of peptide may be required making the proposition less practical, cost effective and, perhaps, safe.

8. Mechanisms of peptide-induced hyporesponsiveness

Peptide activation of T cells and induction of LARs appear to be unrelated to the ensuing immunomodulation observed following intradermal administration. Thus, activation of memory effector T cells in the bronchial mucosa may result in bronchoconstriction whilst presentation of epitopes by quiescent APC (dendritic cells) following trafficking to draining lymph nodes, may be involved in induction of hyporesponsiveness. Following intradermal administration, peptide may enter the peripheral circulation via the cutaneous capillary bed, traffic to the lung and activate effector T cells following rapid, processing-independent presentation by both professional and non-professional APC. Additionally, some material may be taken up by APC and other “sentinel” cells in the periphery (such as immature mucosal dendritic cells) that then migrate to draining lymphoid tissue. The relatively low dose, solubility and inability of the peptides to cross-link allergen-specific antibodies on the surface of antigen presenting cells may render them tolerogenic in this form. In contrast, peptides delivered in small amounts by inhalation may not reach the lymphatics or peripheral circulation in sufficient dose to evoke hyporesponsiveness despite the ability to induce local effector T cell activation in the short term.

9. Conclusions

Challenge of allergic individuals with synthetic T cell peptide epitopes results in immune modulation that may take several distinct forms, depending upon dose and route of administration. Systemic or local delivery of peptides, at the appropriate dose, results in activation of resident mucosal memory effector T cells in an MHC-restricted fashion. T cell activation is transient and results in bronchoconstriction through an, as yet, undefined mechanism. Several days after peptide challenge, antigen-specific hyporesponsiveness is observed and associated with reductions in sensitivity to whole allergen challenge and modulation of PBMC responses *in vitro*. Patterns of PBMC cytokine production appear consistent with the induction of immune regulation. Activation of T cells and the ensuing tolerance are likely to be distinct processes. In conclusion, peptide challenge of allergic individuals

has provided a rapid and safe method with which to study T cell activation and tolerance *in vivo* in man. Findings in this model may inform the study of other chronic inflammatory diseases associated with autoimmunity and transplantation.

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