

Mechanisms of T Cell Peptide Epitope-Dependent Late Asthmatic Reactions

Mark Larché Brigitte M. Haselden William L.G. Oldfield Karen Shirley
Janet North Qiu Meng Douglas S. Robinson Sun Ying A. Barry Kay

Allergy and Clinical Immunology, Imperial College School of Medicine, London, UK

Key Words

T cell · Peptide · Asthma

Abstract

Short peptide sequences corresponding to T cell epitopes have been identified in the major cat allergen Fel d 1. In order to directly activate allergen-specific T cells in cat-allergic asthmatic individuals, peptides were administered by intradermal injection. Subsequently, a proportion of subjects experienced a delayed reduction of airway calibre manifested as a decrease in FEV₁. Changes in lung function occurred approximately 3 h after peptide injection, peaked at 6 h and resembled an isolated late asthmatic reaction (LAR). Using molecular tissue typing techniques, it was determined that many of the individuals experiencing isolated LAR expressed particular HLA-DR molecules. These molecules were shown in subsequent experiments to bind individual peptides within the preparation and thus to activate T cells in a major histocompatibility complex (MHC)-restricted fashion. The precise mechanisms whereby MHC-restricted activation of allergen-specific T cells gives rise to bronchoconstriction are currently under investigation.

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Introduction

Allergic diseases, particularly asthma, are amongst the commonest causes of chronic ill health, are increasing in prevalence and have an appreciable health cost burden. An understanding of immunological mechanisms in allergic asthmatic inflammation in man will extend the base of knowledge, which in turn will lead to measures that will relieve the burden of disease.

A well-established model for studying mechanisms is to provoke asthma in the clinical laboratory under controlled conditions. Thus, following inhalation of allergen there is a decrease in airway calibre occurring within minutes (the early asthmatic reaction) [1]. This acute reaction is generally considered to be due to the release of histamine and other pharmacological agents from mast cells subsequent to cross-linking of IgE by allergen. Three to 8 h after allergen challenge there follows a more prolonged, delayed-in-time, late asthmatic reaction (LAR). In both the skin [2] and the lung [3, 4] the late phase reaction (LPR) is accompanied by an inflammatory cell infiltration, in which eosinophils and Th2 cells are prominent. We and others have provided extensive evidence that CD4⁺ T cells are associated with the LPR in the lung, as well as in chronic asthma [4–7].

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Correspondence to: Dr. Mark Larché
Allergy and Clinical Immunology, Imperial College School of Medicine
National Heart and Lung Institute
Dovehouse Street, London SW3 6LY (UK)
Tel. +44 207 351 8181, Fax +44 207 376 3138, E-Mail m.larche@ic.ac.uk

CD4+ T cell activation is dependent upon presentation of peptide fragments of processed antigen by major histocompatibility complex (MHC) class II on antigen-presenting cells, to specific T cell receptors [8]. Productive T cell responses also require costimulation through such pathways as CD28-CD80/CD86 [9]. Much attention has focused on functional inactivation of such T cell responses leading to 'tolerance'. The absence of costimulation leads to T cell unresponsiveness ('anergy') to rechallenge [10]. In murine models, injection of peptides produced T cell anergy or non-responsiveness through as yet unexplained mechanisms. For example, production of IgG and IL-2 by lymphocytes from Fel d 1-primed mice was decreased after multiple injections of a peptide fragment of the priming allergen [11], and intranasal administration of peptides from Der p 1 could prevent sensitisation and could also inhibit lymphocyte responses in previously sensitised mice [12]. There is good evidence for peptide-induced non-responsiveness of human CD4 cells in vitro [13].

Recently, Norman et al. [14] and Simons et al. [15] attempted to induce the counterpart of murine experimental T cell tolerance [11] in cat-allergic individuals by subcutaneous injection of T cell-reactive peptides (termed IPC1 and IPC2). IPC1 and IPC2 were considerably longer (27 amino acids each) than most previously defined T cell epitopes. This may have been partly responsible for immediate-type (presumed IgE-mediated) hypersensitivity reactions observed in some patients [14]. Although the peptides gave limited protection against natural exposure to cats, large doses ($4 \times 750 \mu\text{g}$) were required to achieve a significant clinical effect [14]. More recently, Muller et al. [16] have demonstrated clinical efficacy in a small group of bee venom-sensitised patients treated with three peptides derived from the sequence of phospholipase A₂.

Materials, Methods and Results

In addition to rapid onset reactions, Norman et al. [14] also observed asthma-like symptoms commencing several hours after administration of IPC1/IPC2. We hypothesised that these reactions were the result of direct, MHC-restricted activation of allergen-specific T cells. To determine whether T cell peptides, which do not cross-link IgE, can induce an isolated LAR, we designed three Fel d 1 chain 1-derived peptides (FC1P) of 16/17 residues and administered them, by intradermal injection, into cat-allergic asthmatic subjects [17].

We recently showed that in humans the LAR could be provoked by direct T cell activation independently of the early IgE response [17]. Intradermal administration of short, allergen-derived peptides directly initiated an MHC-restricted, T cell-dependent LAR, without

the requirement for an early IgE/mast cell-dependent response, in sensitised asthmatic subjects. Unexpectedly, we found that these intradermal injections of T cell peptide epitopes, although producing isolated LAR, had no visible cutaneous response. We believe that this is a unique model for identifying the critical mediator(s) involved in T cell- as opposed to mast cell-dependent airway narrowing. Such observations may have particular relevance to ongoing chronic asthma, which is believed to have an important T cell component but where the sequence of events between T cell activation and bronchial obstruction is incompletely understood.

We established that the peptides, by virtue of their small size, did not release histamine from basophil-enriched mononuclear cells [17]. Following intradermal injection of 80 μg of FC1P, 9 out of 40 cat-allergic asthmatic subjects experienced a fall in FEV₁ which started at 3–4 h and reached a plateau by 6 h [17]. In none of the 40 subjects were immediate lung reactions observed. We hypothesised that these responses represented isolated Th2 cell-dependent LARs. Recognition of antigenic peptides by T cells requires that the peptide be presented to the T cell in the context of an appropriate MHC molecule or 'restriction element'. Since not all individuals developed a response, the HLA-DRB1 haplotype of all subjects was determined [17]. Using allergen-specific T cell lines derived from the study subjects prior to peptide injection and L cells transfected with appropriate HLA-DR alleles, it was determined that peptide FC1P3 could be presented to T cells by both HLA-DRB1*0101 and two microvariants of DR13 (HLA-DRB1*1301 and HLA-DRB1*1302) [17] leading to proliferation and secretion of IL-5. Additionally, another of the three peptides (FC1P2) could be presented by HLA-DRB1*0405 to T cells derived from an autologous (HLA-DRB1*0405) subject and interestingly, to an individual expressing HLA-DRB1*0408.

The recognition of the two peptides in the context of more than one MHC molecule suggests that these peptides are capable of promiscuous binding. This observation may be of some significance, since one argument against the use of peptides for disease-modulating immunotherapy in humans is that because the human population differs widely in their MHC haplotypes, a very large (and therefore impractical) number of peptides will have to be employed in any therapeutic preparation. In fact, what our findings suggest is that individual peptides are capable not only of binding promiscuously to more than one MHC molecule, but that there is also a degree of plasticity at the level of the T cell receptor, since a T cell receptor from an HLA-DRB1*0408 individual can recognise FC1P2 when it is presented in the context of HLA-DRB1*0405. Based on these early observations, therefore, it appears that a relatively small number of carefully selected peptides from an allergen may be efficacious in the treatment of allergic disease. Interestingly, similarly promiscuous peptides have been identified in other allergens such as Bet v 1 [18] and bee venom phospholipase A₂ [19].

Thus, it was possible to account for FC1P-mediated induction of LAR by virtue of the 9 individuals expressing a DR1, DR4 or DR13 allele. These findings strongly support the hypothesis that direct, MHC-restricted activation of Th2 lymphocytes by allergen-derived peptides leads to the development of the LAR suggesting that both the Th2 component of asthma and strategies to downregulate this response are founded firmly in MHC-restricted Th2 cell recognition of allergen epitopes.

Discussion

A number of possibilities exist to explain the mechanism underlying T cell-dependent induction of bronchoconstriction. One possibility is that T cell activation, in and around the airways, leads to the mobilisation of eosinophils through the elaboration of type 2 and eosinophil-active cytokines [e.g. interleukin-3 (IL-3), IL-4, IL-5 and granulocyte macrophage-colony stimulating factor]. These cytokines, together with CC chemokines, especially eotaxin, lead to selective eosinophil accumulation, migration and activation. This, in turn, promotes bronchial smooth muscle contraction and airway oedema through the generation of eosinophil membrane-derived sulphido-peptide leukotrienes [20]. An alternative possibility would be the elaboration, by T cells, of histamine-releasing factors which lead to mast cell degranulation. The role of such mechanisms in T cell-dependent isolated LAR is currently under investigation.

A further alternative may be found in recent studies which have raised the possibility that those elements of allergic airway inflammation which involve smooth muscle contraction, submucosal oedema and mucous hypersecretion may be due to local neuropeptide release, consequent to the elaboration of neurotrophins [21]. Thus, one hypothesis is that sensitised T cells, activated by T cell peptide epitopes, elaborate NGF and other neurotrophins which bind to specific receptors (called *trk*) in and around sensory neurones. These are internalised and retrogradely transported to the neuronal cell body. In the nucleus they induce transcriptional activation leading to neuronal hyperreactivity and enhanced neuropeptide synthesis. These neuropeptides are then transported into the periphery to be released upon stimulation by antidromic mechanisms. A second possibility is that T cell activation results in stimulation of sensory nerve fibres in the submucosa. This may be effected by release of as yet unidentified soluble factors, or by direct T cell interaction with the fibre. Sensory nerve impulses would then be received in the nerve ganglion and give rise to cholinergic drive leading to smooth muscle contraction and a reduction in airway calibre.

There may also be direct interactions between T cells, or their products, and airway smooth muscle (ASM) leading to contraction. T cells are known to adhere directly to ASM cells via integrins and CD44 [22], and TNF- α , a known product of activated T cells and present in BAL fluid in asthmatics [23], induces calcium mobilisation and electrophysiological changes in ASM cells [24]. ASM cells are a rich source of inflammatory mediators, includ-

ing CC chemokines and pro-inflammatory cytokines [25] which on the one hand, can be induced by agents such as TNF- α , and on the other, could amplify T cell peptide-induced airway narrowing. There is also strong evidence from experimental animals that IL-13 may directly induce airway hyperreactivity, by a mechanism which, like peptide-induced LAR, is independent of IgE [26, 27].

Conclusions

The clinical model described provides a powerful system in which to investigate the precise role of the T cell in late-phase allergic inflammation. It allows the confounding influence of the early phase reaction to be removed, and in so doing, allows the identification of the components of the LPR which are dependent upon a preceding early phase reaction and those which are not. The ability to dissect the cellular mechanisms of the complex events which give rise to allergic reactions, and lead to chronic allergic inflammation, will lead to a better understanding of how therapeutic approaches may be designed in order to establish regulation of the cellular mechanisms which drive allergic diseases.

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