Airway hyperresponsiveness and bronchial mucosal inflammation in T cell peptide-induced asthmatic reactions in atopic subjects

F Runa Ali, A Barry Kay, Mark Larché

Background: Subjects with allergic asthma develop isolated late asthmatic reactions after inhalation of allergen-derived T cell peptides. Animal experiments have shown that airway hyperresponsiveness (AHR) is CD4+ cell-dependent. It is hypothesised that peptide inhalation produces increases in non-specific AHR and a T cell-dependent bronchial mucosal response.

Methods: Bronchoscopy, with bronchial biopsies and bronchoalveolar lavage (BAL), was performed in 24 subjects with cat allergy. Biopsy specimens and BAL fluid were studied using immunohistochemistry and ELISA.

Results: Twelve of the 24 subjects developed an isolated late asthmatic reaction with a preceding early (mast cell/histamine-dependent) reaction characteristic of whole allergen inhalation. These responders had significant between-group differences (responders vs non-responders) in the changes (peptide vs diluent) in AHR (p = 0.007) and bronchial mucosal CD3+ (p = 0.005), CD4+ (p = 0.006) and thymus- and activation-regulated chemokine (TARC) (p = 0.003) but not CD8+ or CD25+ cells or eosinophils, basophils, mast cells, and macrophages. The between-group difference for neutrophils was p = 0.05 but with a non-significant within-group value (peptide vs diluent, responders, p = 0.11). In BAL fluid there was no significant between-group difference in TARC (p = 0.02) but not in histamine, tryptase, basogranulin, C3a or C5a, leukotrienes C4/D4/E4, prostaglandins D2 or E2.

Conclusions: Direct activation of allergen-specific airway T cells by peptide inhalation in patients with atopic asthma leads to increased AHR with local increases in CD3+ and CD4+ cells and TARC but no significant changes in eosinophils or basophil/mast cell products. These findings support previous animal experiments which showed a CD4+ dependence for AHR.

Abbreviations: AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; CGRP, calcitonin gene-related peptide; FEV1, forced expiratory volume in 1 s; histamine PC20, histamine provocative concentration; IL, interleukin; LAR, late asthmatic reaction; LT, leukotriene; MBP, major basic protein; PEFR, peak expiratory flow rate; PG, prostaglandin; TARC, thymus- and activation-regulated chemokine
tion of either *Fel d* 1-derived peptides or diluent control. The pharmacological mediators included known bronchoconstricting agents (eg, histamine and eicosanoids), the histamine-releasing complement-derivated anaphylotoxins C3a and C5a, as well as markers of mast cell degranulation (tryptase and basogranulin). We also measured interleukin (IL)-13 in lavage fluid as this cytokine is known to be associated with increased AHR. Interleukin-10, a regulatory cytokine, was also assayed to determine whether it was altered in responders compared with non-responders.

**METHODS**

**Subjects and study design**

Volunteers with asthma who were allergic to cats were recruited by advertisement and characterised clinically as defined previously. The study was approved by the Royal Brompton and Harefield NHS Trust ethics committee. All volunteers gave written informed consent. All subjects had a PC_{20} to histamine of <16 mg/ml at screening, evidence during the previous 12 months of >15% reversibility of the FEV_{1} or peak expiratory flow rate (PEFR) either spontaneously or after inhaled β_{2} agonists, and a clear history of wheezy breathlessness with or without cough on exposure to cats. β_{2} agonists were withheld on the study day and inhaled corticosteroids were discontinued 2 months before entering the study. Subjects were excluded if they had received oral corticosteroids in the previous 2 months or *Fel d* 1-derived peptides in the preceding 6 months. Subjects were non-smokers and had no history of current illness or clinically significant abnormalities in routine haematology, biochemistry or urinalysis.

A randomised, placebo-controlled, crossover study design was used. Seven days after screening (visit 1), subjects received either nebulised diluent (0.9% saline) or 5 μg *Fel d* 1-derived peptide (12 overlapping peptides from chains 1 and 2 of *Fel d* 1). In all instances, subjects were unaware of whether they were inhaling peptides or diluent. The challenge was postponed if the baseline FEV_{1} fell below 80% predicted on any study day. To exclude significant worsening of an individual’s hyperresponsiveness, the nebulised peptide challenge was administered only if the subject did not exhibit a decrease in FEV_{1} of ≥10% to an initial inhaled control (diluent) challenge. The FEV_{1} was then recorded at 0, 15, 30 and 60 min and hourly thereafter for 4 h, at which time bronchoscopy with bronchial biopsies and BAL was performed. Seven days later (visit 3) the histamine PC_{20} was measured. On visit 4 (minimum of 4 weeks after visit 2) volunteers again inhaled either diluent or peptide (ie, the opposite of what was given on visit 2) and bronchoscopy with biopsies and BAL was again performed. One week later (visit 5) the histamine PC_{20} was repeated.

Thirty-one subjects entered the study but only 12 developed an isolated LAR (>20% reduction in FEV_{1}) to peptide. These responders completed both the control and peptide study days. The first 12 subjects who showed no clinical response (non-responders) to peptide also underwent both challenges and bronchoscopies. The remaining seven non-responders were not investigated since an equal number of subjects in each investigational group (12 responders and 12 non-responders) had been attained.

**Peptide synthesis and validation**

Twelve overlapping peptides from chains 1 and 2 of *Fel d* 1 (chain 1: EICPAVKRDVDLFLTGT, LFLTGTPDEYVEQVAQY, EQVAQYKALPVVLENA, KALPVVLENARLKNVCY, RILKNVCYD-PKTEEDSleep, KEMKTEEDKENALSLDLDK, KENALSLDKIYSGSPL; chain 2: LTKVYNATEPERTAMKK, TAMKKIQQCICYVENGLI, SRLDGLVMTTITSSSK, ISSSSKDCMEAVONTV, AVONTVED-LKNTLGR) were synthesised and dispensed as described. These were previously shown not to release histamine from peripheral blood basophils.

**Inhalational challenge**

The peptide solution was diluted to 1 ml with 0.9% saline and delivered through the Pari LC Star nebuliser plus filter and Pari Boy compressor (Pari Medical Ltd, West Byfleet, UK) for 10 min.

**Fibreoptic bronchoscopy**

Fibreoptic bronchoscopy with BAL and bronchial biopsies was performed 6 h after inhalational challenge of either diluent control or *Fel d* 1 peptides, as described in detail elsewhere. BAL fluid and cells and biopsies were also processed as described previously.

**Immunohistochemistry**

Cryostat sections (6 μm) were freshly cut from biopsy specimens, mounted on 0.1% poly-L-lysine coated slides and air dried overnight at room temperature. Monoclonal antibody staining was assessed by the alkaline phosphatase anti-alkaline phosphatase method as previously described. Normal human serum (10%) was used to prevent non-specific binding of the second and third layer antibodies. A mouse IgG_{1} myeloma protein was used as a negative control. The monoclonal antibodies used were CD3, CD4, CD8, CD68, neutrophil elastase, human mast cell tryptase (Dako, High Wycombe, UK); CD25 (Becton Dickinson, Cowley, Oxford, UK); major basic protein (MBP) (BMK 13; in-house); TARC (R&D Systems, Minneapolis, USA) and BB1, a monoclonal antibody which recognises a human basophil granular protein (a gift from Dr A F Walls, University of Southampton, UK). Polyclonal rabbit anti-mouse immunoglobulin and APAAP reagents were purchased from DAKOPATTS (Dako, Ely, UK). For TARC staining, cryostat sections (5 μm) were mounted on Superfrost Plus slides (VWR, UK) and air dried overnight at room temperature. TARC was identified using a Vectastain ABC Kit (Vector Laboratories, Peterborough, UK). The reaction was visualised using Fast Red Substrate (Vector Laboratories). The TARC antibody used was raised in the goat (R&D Systems, Oxford, UK). The numbers of positively stained cells were counted in a zone 250 μm deep along the entire length of epithelial basement membrane, as defined by a squared eyepiece graticule. Cell counts were performed in a blinded fashion and expressed as the number of positive cells per mm^{2}.

**Differential BAL cell counts**

Cells counts were performed in a blinded fashion. Unfixed cytospins of BAL cells were stained using Kwik-Diff. The slides were rinsed in phosphate-buffered saline and air dried. In all cases a minimum of 400 nucleated cells were counted per slide.

**Histamine measurement**

The histamine content of BAL supernatant and following stimulation of whole blood by BAL was measured by means of a commercially available competitive ELISA kit (Immunotech, Marseilles, France) according to the manufacturer’s instructions.

**Eicosanoid assays**

BAL supernatants were frozen immediately and stored at −80°C until further analysis. Thawed BAL supernatant (5 ml) was purified and concentrated 10-fold on a C18 column. The recovery of prostaglandins (PGs) and leukotrienes (LTs) was determined by the addition of 3H-PGD_{2} (10 000 cpm; Amersham Bioscience UK Ltd, Bucks, UK) and 3H-LTC_{4}
Table 1 Clinical characteristics of subjects with asthma and cat allergy

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<th>Responders (n = 12)</th>
<th>Non-responders (n = 12)</th>
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<tr>
<td>Ratio M:F</td>
<td>4:8</td>
<td>6:6</td>
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<tr>
<td>Age (years)</td>
<td>27 (26–34)</td>
<td>28 (22.5–28.5)</td>
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<tr>
<td>Baseline FEV1 (% pred)</td>
<td>93.8 (87.5–102.7)</td>
<td>92.8 (86.1–95.6)</td>
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<td>PC20 histamine (mg/ml)</td>
<td>3.99 (1.67–12.5)</td>
<td>3.99 (1.93–4.63)</td>
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<tr>
<td>Total IgE (IU/ml)</td>
<td>134 (96–248.5)</td>
<td>256.5 (197–567.4)</td>
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<tr>
<td>Cat RAST (IU/ml)*</td>
<td>29.2 (6.17–43.9)</td>
<td>3.49 (1.98–7.07)</td>
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| FEV1%, forced expiratory volume in 1 s; PC20 histamine, concentration of histamine provoking a fall in FEV1 of 20% or more. | *p = 0.002. |

(6000 cpm; NEN Life Science Products, Hounslow, UK), respectively, to each 5 ml aliquot. LTC4, LTE4 and 11β-PGF2α in the concentrated samples were measured by enzyme immunoassay (Cayman Chemical Co, An Arbor, Michigan, USA). PGD2 in the concentrated samples was converted to PGD2-methoxime (PGD2-MOX), a stable derivative, which was measured by enzyme immunoassay (Cayman Chemical Co). The specificity of the antibody to 11β-LTE4 is 100% for 11β-LTE4 and 5.5 pg/ml, PGD2-MOX 3.6 pg/ml.

The specificity of the LTC4 antibody is 100% for LTC4 and LTC5 and the antibody cross-reacts with LTD4 (10%) and LTD5 (7%). The specificity of the LTE4 antibody is 100% for LTE4 and LTE5, with cross-reactivity with LTC4 (10%) and LTD4 (7%). The specificity of the antibody to 11β-PGF2α is 100% for 11β-PGF2α, 10% for 2,3-dinor-11β-PGF2α and <0.01% for LTs, thrombomodulin B2 and other PGs. The antibody to PGD2-MOX is specific for PGD2-MOX (100%), with cross-reactivity with PGD2 (0.2%) and other PGs (<0.01%).

ELISAs for cytokines

Aliquots of 10 ml BAL supernatant were thawed and immediately concentrated 10 times using Amicon Centrulites kit (Millipore, Billerica, Massachusetts, USA) according to the manufacturer’s instructions. The levels of IL-10 and IL-13 in the concentrated BAL supernatants were determined by ELISA (PelliKine Compact kit, CLB, Amsterdam, The Netherlands). The sensitivity of the assays is 1–3 pg/ml for IL-10 and 0.5–1.5 pg/ml for IL-13.

Tryptase and basogranulin measurements

Tryptase measurement was performed using a modification of a previously described ELISA method14 that detects both protryptase and the mature forms (with a sensitivity of 0.5 ng/ml). Basogranulin assay was performed using a dot blotting procedure with monoclonal antibody BB1 as described previously.15 Concentrations were expressed as milliunits/ml, where 1 unit was taken as the amount of basogranulin in a standard preparation of purified basophil.

C3a and C5a assays

Levels of C3a/C3a des-arginine and C5a/C5a des-arginine in BAL supernatants were determined by ELISA using BD Biosciences Pharmingen kits (San Diego, USA) according to the manufacturer’s instructions. In order to prevent ex vivo complement activation, FUT-173 (Futhan, final concentration 5 μg/ml; BD Biosciences, #52035) was added to each thawed sample. Samples were assayed undiluted, 1:3, 1:6, 1:12 as previously described.16 The sensitivities of the assays are 7.3 pg/ml for C3a and 0.06 ng/ml for C5a.

TARC assay

The TARC level in BAL supernatants was determined by ELISA (R&D Systems, Abingdon, UK) according to the manufacturer’s instructions. The sensitivity of the assay was 7 pg/ml.

BAL fluid assays: protein correction factor

BAL fluid samples were corrected for variable dilution using protein as an internal reference standard. Protein determination in BAL fluid and concentrated BAL supernatants was performed using bichinchoninic acid protein assay kit (Sigma, St Louis, USA) according to the manufacturer’s instructions. The levels of IL-10 and IL-13 in the concentrated BAL supernatants were determined by ELISA (PelliKine Compact kit, CLB, Amsterdam, The Netherlands). The sensitivities of the assays is 1–3 pg/ml for IL-10 and 0.5–1.5 pg/ml for IL-13.

Figure 1 Late asthmatic reactions and airway hyperresponsiveness (AHR) in peptide responders and non-responders. Change in forced expiratory volume in 1 s (FEV1) after peptide challenge (closed circles) and diluent challenge (open circles) in (A) 12 responders and (B) 12 non-responders. Differences in the area under the curve between the control day and peptide day were analysed by paired t test. (C) Changes in AHR in responders and non-responders 7 days after inhaled challenge. PC20, concentration of histamine provoking a fall in FEV1 of 20% or more; D, diluent inhalation; P, peptide inhalation. Bars represent median values. Within-group comparisons of AHR after diluent and peptide inhalation were performed using two-tailed Wilcoxon signed rank tests. Between-group comparisons of the change from diluent to peptide in responders and non-responders were performed using the non-parametric Mann-Whitney test.
Mann-Whitney test was also used for unpaired data. Correlations with clinical characteristics were performed using Spearman’s rank coefficient of correlation. FEV₁ data were summarised over time for each subject for the control (diluent) day and the peptide day. Areas under each curve for the FEV₁ measured over 6 h were calculated using the trapezoidal rule. Differences in the areas under the curves between the control day and peptide day were analysed by paired t test.

RESULTS
Effect of peptide challenge on AHR
At baseline, responders and non-responders were well matched for sex, age, FEV₁, histamine PC₂₀ and total serum IgE concentration (table 1). The changes in FEV₁ following inhalation of either peptide or diluent in responders and non-responders are shown in fig 1A and B, respectively. On the peptide day, responders had a mean decrease in FEV₁ at 6 h of approximately 33% (p<0.001) while, on the diluent day, there was virtually no change between FEV₁ at baseline and after 6 h in responders. No changes in FEV₁ occurred on diluent or peptide days in non-responders. There was a highly significant increase in cat-specific serum IgE in responders compared with non-responders despite a non-significantly lower concentration of total IgE (p = 0.002, table 1). Changes in AHR are shown in fig 1C. There was a significant decrease in the histamine PC₂₀ in responders (p = 0.002 diluent vs peptide) which was not observed in non-responders (p = 0.52 diluent vs peptide). The between-group difference in the changes was p = 0.007. On entering the study, all subjects had mild asthma with a history of cat-induced wheeze. At screening they all had a PC₂₀ histamine of <16 mg/ml. However, in two of the subjects the values changed to >16 mg/ml during the course of the study (fig 1C).

Association of isolated LARs with recruitment of CD3⁺CD4⁺ T cells
In responders there were significant increases (diluent vs peptide) in the numbers of CD3⁺ (p = 0.05) and CD4⁺ cells (p = 0.03; fig 2 and table 2), with decreases in non-responders (p = 0.03 for CD3⁺ and p = 0.09 for CD4⁺ cells, table 2). The between-group differences in the changes were p = 0.005 for CD3⁺ cells and p = 0.006 for CD4⁺ cells (fig 2 and table 2). Using a non-paired test, the numbers of CD3⁺ and CD4⁺ cells were also significantly higher in responders than in non-responders following peptide challenge (p = 0.004 and p = 0.04, respectively), but not following inhaled diluent (fig 2). A representative photomicrograph of CD4⁺ immunostaining in bronchial biopsy specimens from responders and non-responders is shown in fig 3. In responders (diluent vs peptide) there was only a trend for increases in MBP⁺ eosinophils (p = 0.07, table 2). Although there was no significant within-group increase in elastase⁺ neutrophils (p = 0.11) in responders (diluent vs peptide), the between-group value just reached significance (p = 0.05). There were no significant changes in CD8⁺ and CD25⁺ cells or in BB1⁺ basophils or tryptase⁺ mast cells (table 2).

Eosinophil and neutrophil counts in BAL fluid showed no significant change in either responders or non-responders (diluent vs peptide). The p values for between-group differences were 0.44 and 0.37 for eosinophils and neutrophils, respectively (data not shown).

Association of isolated LARs with increased expression of the Th2 chemokine TARC
There was a significant increase in the concentration of TARC in BAL fluid (diluent vs peptide) in responders (p = 0.004) but not in non-responders (p = 0.97) with a between-group difference.

Statistical analyses
Statistical analyses were performed using commercial software packages including GraphPad Prism and p levels of ≤0.05 were considered significant. Data from BAL fluid supernatant assays, BAL cytospins, bronchial biopsy immunohistochemistry and in situ hybridisation analyses are expressed as medians with minimum to maximum ranges. Statistical comparisons of diluent and peptide inhalation (within-subject comparisons) were performed using non-parametric two-tailed Wilcoxon signed rank tests. Between-group comparisons of the change from diluent to peptide in responders and non-responders were performed using the non-parametric Mann-Whitney test.
of $p = 0.02$ (fig 4A). However, as shown by the non-paired test, the concentrations of TARC were significantly higher in non-responders than in responders following diluent ($p = 0.03$) but not following peptide challenge (fig 4A).

The results for TARC+ immunoreactive cells in bronchial biopsy specimens are shown in fig 4B. There was a significant increase in TARC+ cells (diluent vs peptide) in responders ($p = 0.005$) but not in non-responders ($p = 0.68$), with a between-group difference of $p = 0.003$. However, unlike BAL fluid, there was no significant difference in TARC+ cells after diluent challenge but a significant increase after peptide ($p = 0.02$, fig 4B). TARC+ cells included eosinophils, neutrophils and mononuclear cells. There was also increased positive TARC staining in epithelial cells in responders but this was not formally quantified. There was a positive correlation between the magnitude of the LAR (expressed as area under the curve) and the delta changes (diluent vs peptide) in the 12 responders for TARC+ cells in biopsy specimens ($r = 0.59$, $p<0.05$). A representative photomicrograph of TARC+ immunostaining in bronchial biopsy specimens from responders and non-responders is shown in fig 3.

**Effect on BAL fluid levels of pharmacological mediators, IL-13 and IL-10**

The concentrations of various mast cell- and basophil-associated pharmacological mediators in BAL fluid are shown in table 3. There were no significant differences (responders vs non-responders, diluent vs peptide) in histamine, tryptase,
basogranulin, LTC4/LTD4/LTE4 PGD2, PGF2α and fragments cleaved from C3 (C3a). C5a was not detectable in any sample. There were also no significant increases (diluent vs peptide) in the concentrations of IL-13 or IL-10 in BAL fluid from responders or non-responders (data not shown).

DISCUSSION

The important novel observation of this study is that, in subjects with asthma, selective activation of allergen-specific T cells by inhalation of allergen-derived peptides is sufficient to induce increases in non-specific AHR (a cardinal feature of asthma) which, in turn, is accompanied by a predominantly T cell bronchial mucosal inflammatory response. Thus, in responders but not in non-responders, inhalation of peptides produced increased AHR (measured 7 days after peptide provocation) as well as a LAR (which peaked at 6 h after challenge) as previously described.12

The increased numbers of CD3+, CD4+ and TARC+ cells in biopsy specimens supports the view that increased AHR is linked to T cell activation and are in keeping with previous animal studies. For example, adoptive transfer experiments in Brown Norway rats have shown that AHR can be transferred by allergen-specific CD4+ T cells.8 Moreover, in mice, using a combination of anti-T cell monoclonal antibody, T cell transfer and bone marrow transplantation, it was shown that T cells enhanced genetically determined AHR.7 Furthermore, depletion of murine CD4+ T lymphocytes prevented antigen-induced AHR and pulmonary eosinophilia.10

In a previous study14 we examined bronchial biopsies and BAL fluid from responders and non-responders 6 h after an intradermal injection of allergen-derived peptides (ie, when airway narrowing was maximal). Surprisingly, we found no changes (diluent vs peptide challenge) in the numbers of eosinophils, neutrophils, basophils, mast cells, T cells and T cell subsets, macrophages, Th2 cytokines, histamine, histamine-releasing factors or eicosanoids. However, as the challenge route was systemic, T cell activation may have occurred in perivascular tissue distal to bronchoscopic sampling. In the present study peptides were delivered via the inhaled route using a method previously reported,2 so we anticipated that, with direct airway challenge, we were more likely to observe mucosal infiltration of inflammatory cells and changes in concentrations of pharmacological mediators. In fact, with the exception of increases in airway T cells, our findings using the inhaled route were very similar to those observed with LARs provoked by intradermal challenge.14 For example, no increases...
were observed in the BAL fluid concentrations of tryptase, basogranulin, C3a, C5a, LTC₄/DLT₂E₄, PGD₂ or PGE₂ between diluent and peptide in either responders or non-responders. With the exception of basogranulin, these mediators have all been shown to be increased in BAL fluid following whole allergen challenge.

Although there was an increase in neutrophils and a trend for an increase in eosinophils, there were no significant differences in the numbers of basophils or macrophages. This is in contrast to changes induced in the airways after challenge with whole allergen. For example, in our previous study in which we measured cells and mediators in the airways 6 h after whole allergen challenge, there were increases in bronchial eosinophils, neutrophils, T cells, histamine and LTC₄ in dual but not single early responders. Others have also observed whole allergen-induced airway eosinophilia in subjects with mild asthma at the 6 h time point. Taken together, these studies support the view that eosinophil infiltration is more likely to be secondary to mast cell rather than T cell activation, and therefore might not occur following T cell peptide inhalation since there is a lack of IgE cross-linking on mast cells. Furthermore, the role of the eosinophil in allergen-induced late reactions has been cast in doubt since depletion of this cell with anti-IL-5 had no effect on either late phase asthmatic or skin reactions. Nevertheless, we cannot completely exclude the role of eosinophils and neutrophils in peptide-induced LARs, especially as the study population was small. However, taken together with our previous biopsy and lavage study, our data would suggest that they are not crucial cells in the pathogenesis of the peptide-induced late phase reaction.

An unexpected finding was that the concentrations of TARC were significantly higher in non-responders than in responders following diluent challenge (fig 4A) as shown by a non-paired t-test (p = 0.03). In fact, this was in large part due to two subjects who had a particularly high response to diluent challenge. The fact that the more meaningfully paired within-group and between-group data showed significance with BAL fluid and that there was a very clear TARC immunoreactive positive cell response at the level of the bronchial mucosa (fig 4B) suggests that changes in this Th2 chemokine are central and relevant in our model of asthma.

A further novel finding was that at baseline (ie, before challenge) the responders had significantly raised serum cat-specific IgE compared with non-responders. It has previously been shown that dual responders (those who develop early and late asthmatic reactions and subsequent increased AHR after whole allergen challenge) tended to have raised allergen-specific IgE compared with single early responders. This observation may help to predict why, some, but not all, cat-sensitive individuals develop a LAR after the Fel d 1 peptide challenge. Thus, raised levels of allergen-specific serum IgE may reflect high IgE density on airway dendritic cells and/or the frequency of allergen-specific T cells in the airway mucosa. Further experiments will be required to test these hypotheses. It should be emphasised that the present study supports the view that increased AHR occurs after a LAR irrespective of whether this is induced by whole allergen (and therefore preceded by a mast cell-dependent early response) or is an isolated peptide-induced LAR as in the present study. In both situations (whole allergen or peptide challenge) the subjects who develop a LAR have raised serum levels of allergen-specific IgE. Thus, T cell activation rather than mast cell activation may be more crucial for the development of increased AHR as previously observed in animal studies.

In summary, the following mechanism is proposed. Inhaled T cell peptide epitopes bind to MHC class II molecules expressed in the airways leading to activation of peptide-specific resident effector memory T cells. Activation of these cells enhances local production of TARC from antigen-presenting cells, structural cells and accessory cells. Increased TARC expression leads to recruitment and activation of CD3⁺/CD4⁺ T cells, production of inflammatory cytokines (although this has yet to be demonstrated) and, ultimately, increased AHR. Our inability to demonstrate cells mRNA+ for Th2 cytokines in 6 h biopsy specimens may have been related to the small numbers of IL-4, IL-5 and IL-13 positive cells at this time point (as opposed to 24 h where previously we have found increases after whole allergen challenge). The mechanism of the late-phase reaction itself remains uncertain but, in a recent report, we showed markedly raised expression of calcitonin gene-related peptide (CGRP) in epithelial cells, infiltrating CD3⁺/CD4⁺ cells and smooth muscle in responders (but not non-responders) after peptide challenge. Since CGRP is a potent vasodilator, this raises the possibility that airway wall oedema may be an important component of peptide-induced late reactions. In any case, the present findings support the view that T cell activation alone is capable of triggering these events, observations which may be of importance to unravelling events in chronic severe asthma where the role of the T cell is now well established.

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REFERENCES


LUNG ALERT

What factors are predictive of survival in patients with non-small-cell lung cancer treated with gefitinib?

This study identified factors associated with prognostic benefit from gefitinib chemotherapy. Japanese patients who had received gefitinib monotherapy between 2002 and 2005 at the Hyogo Medical Centre for Adults in Japan (n = 221) were included in the study. Their clinical parameters were retrospectively examined for potential predictive factors of survival. Median survival time was better in females, 13.3 vs 6.8 months (p = 0.036); patients with adenocarcinoma, 9.3 vs 3.6 months (p = 0.137); never smokers, 14.5 vs 6.5 months (p < 0.001); those with favourable performance status, 11.1 vs 2.1 months (p < 0.001); and patients with epidermal growth factor receptor (EGFR) mutation, 24.9 vs 7.4 months (p < 0.001). The lower the smoking exposure (Brinkman Index: cigarettes per day × years smoked) the longer the mean survival time (p < 0.001). Multivariate analysis showed that positive EGFR mutation status and performance status 0–1 were independent predictors of a favourable prognosis. Prognosis was significantly different according to EGFR mutation status (with the same smoking status), but not according to smoking status (with the same EGFR mutation status). The authors suggest that although smoking is not a direct predictor of prognosis, it may be useful as a surrogate marker for EGFR mutation status. They concluded that EGFR mutation status is the most important independent predictor of survival benefit with gefitinib treatment.

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