

Original article

Airway expression of calcitonin gene-related peptide in T-cell peptide-induced late asthmatic reactions in atopics

Background: The mechanisms of late asthmatic reactions provoked in atopic asthmatics by allergen-derived T-cell peptide epitopes remain unclear. Previous studies showed no changes in airway eosinophils or mast cell products after peptide challenge. In the present study our aim was to measure calcitonin gene-related peptide (CGRP), neurokinin (NK)-A, and substance P (SP) in bronchoalveolar lavage fluid and bronchial biopsies (BB) after inhalation of allergen-derived T-cell peptide epitopes since these neuropeptides (NP) had not previously been evaluated in this chronic asthma model.

Methods: Bronchoscopy, with BB and bronchoalveolar lavage (BAL), was performed in 24 cat-allergic subjects 6 h after inhalation of Fel d 1-derived peptides. Neuropeptides were measured in BAL by enzyme-linked immunosorbent assay and CGRP expression in the airways was assessed by immunohistochemistry and confocal microscopy.

Results: Twelve subjects (termed 'responders') developed isolated late reactions. Calcitonin gene-related peptide, but not NK-A or SP, was significantly elevated in BAL in responders only. Biopsy studies showed that in virtually all responders peptide challenge induced marked increases in CGRP immunoreactivity in bronchial epithelial cells, infiltrating submucosal cells and in association with airway smooth muscle. Double immunostaining indicated that CGRP colocalized predominantly to CD3+/CD4+ and CD68+ submucosal inflammatory cells.

Conclusion: Calcitonin gene-related peptide, a potent vasodilator, is markedly up-regulated in the airways of atopic asthmatics during late-phase reactions provoked by inhalation of allergen-derived T-cell peptides.

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Early- and late-phase asthmatic reactions (EAR, LAR) provoked by allergen inhalation are useful models for studying mechanisms of airway narrowing. The EAR is generally believed to result from the release of histamine and other products as a consequence of mast cell activation. The precise nature of the mediator(s) respon-

sible for late-phase allergic responses remains uncertain, although previous data suggested that these are generated, in part, as a result of T-cell activation (1).

We have previously shown that allergen-derived T-cell peptide epitopes, administered by either intradermal injection (2) or by inhalation (3), induce LARs in a proportion of atopic asthmatics (responders), but not in others (nonresponders). These late-phase reactions peaked between 3 and 9 h after peptide inhalation and had a similar time-course of onset and resolution to LARs induced by whole allergen extract. They are termed 'isolated' late reactions as there was no early (immediate) asthmatic reaction, presumably because the peptides were too short to cross-link IgE on mast cells and basophils. Thus our model has the advantage of providing information on the T-cell component of allergic airway inflammation, independently of initial mast cell activation.

Abbreviations: AHR, airway hyperreactivity; APAAP, alkaline phosphatase anti-alkaline phosphatase; BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; BB, bronchial biopsy; cAMP, cyclic adenosine monophosphate; CGRP, calcitonin gene-related peptide; COPD, chronic obstructive pulmonary disease; EAR, early asthmatic reaction; FEV₁, forced expiratory volume in one second; LAR, late-phase asthmatic reaction; NEC, neuroendocrine cells; NK, neurokinin; NP, neuropeptide; PC₂₀, histamine provocative concentration causing a 20% fall in FEV₁; RIA, radioimmunoassay; SP, substance P; VEGF, vascular endothelial growth factor.

We previously showed, using intradermal peptide challenge, that there were no increases in bronchoalveolar lavage (BAL) in the concentrations of a wide range of pharmacological mediators (including histamine, leukotrienes, prostaglandins, and neurotrophins) or in the numbers of eosinophils, neutrophils, and basophils in the airway wall (4). For this reason we speculated that neuropeptides (NP) may be operative in events leading to airway narrowing in this model as it is well documented that these have profound effects on both vascular leakage and airway smooth muscle contraction (5). Using this approach we have found that, after inhaled peptide challenge, responders, but not nonresponders, had high expression of the potent vasodilator, calcitonin gene-related peptide (CGRP) [but not neurokinin-A (NK-A) or substance P (SP)], in bronchial biopsies (BB), and bronchoalveolar lavage fluid (BALF) recovered at 6 h, i.e. when the LAR was well established. One of the implications of this novel finding is that it refocuses attention on the role of the vasculature in these delayed-in-time allergic airway responses.

Methods

Subjects & study design

Cat-allergic asthmatic volunteers were recruited and characterized clinically as defined previously (2,3). The study was approved by the Royal Brompton and Harefield NHS Trust Ethics Committee. All volunteers gave written informed consent. Some of the results from this study have been reported elsewhere (6). All subjects demonstrated a histamine provocative concentration causing a 20% fall (PC_{20}) in forced expiratory volume in one second (FEV_1) to histamine of ≤ 16 mg/ml at screening, evidence, during the previous 12 months, of more than 15% reversibility of the FEV_1 or peak expiratory flow rate, either spontaneously or after inhaled β_2 -agonists and a clear history of wheezy breathlessness with, or without, cough on exposure to cats. The 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.

Seven days after screening (visit 1), subjects received either nebulized diluent (0.9% saline) or 5- μ g 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. The nebulized peptide challenge was administered only if the subject did not exhibit a decrease in $FEV_1 \geq 10\%$ to an initial inhaled control (diluent) challenge (thereby excluding nonspecific bronchial hyperresponsiveness). The FEV_1 was then recorded at 0, 15, 30, and 60 min and hourly thereafter for 5 h at which time bronchoscopy with BB 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 (i.e. the opposite of what was given on visit 2) and bronchoscopy with biopsies and lavage was again performed. One week later (visit 5) the histamine PC_{20} was repeated.

Thirty-one people entered the study but only 12 subjects developed an isolated late asthmatic reaction ($> 20\%$ 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 (nonresponders) to peptide also underwent both challenges and bronchoscopies. The remaining seven nonresponders were not investigated further as they did not have complete BAL/biopsy data pair sets and equal number of subjects in each investigational group (12 responders and 12 nonresponders) had been attained.

Peptide synthesis and validation

Twelve overlapping peptides from chains 1 and 2 of Fel d 1 (chain 1: EICPAVKRDVDLFLTLGT, LFLTGTPEYVEQVAQY, EQVAQYKALPVVLENA, KALPVVLENARILKNCV, RILKNCVD-AKMTEEDKE, KMTEEDKENALSLLDK, KENALSVLDDKI-YTSPL and chain 2: LTKVNATEPERTAMKK, TAMKKIQDC-YVENGLI, SRVLDGLVMTTSSSK, ISSSKDCMGEAVQNTV, AVQNTVEDLKLNTLGR) were synthesized and dispensed as described (7). These were previously shown not to release histamine from peripheral blood basophils.

Inhalational challenge

The peptides solution was diluted to 1 ml with 0.9% saline and delivered through the Pari LC Star nebulizer plus filter and Pari Boy compressor (Pari Medical Ltd., West Byfleet, UK) for 10 min (3).

Fibreoptic bronchoscopy

Fibreoptic bronchoscopy with BAL and BB was performed 6-h following inhalational challenge of either diluent control or Fel d 1 peptides, as described in detail elsewhere (8). Bronchoalveolar lavage fluid and cells and biopsies were also processed as described previously (8).

Immunohistochemistry

Cryostat sections (6 μ m) were freshly cut from biopsies, mounted on 0.1% poly-L-lysine coated slides and air dried overnight at room temperature. Monoclonal antibody staining was detected by alkaline phosphatase anti-alkaline phosphatase (APAAP) method, as previously described. (9). Normal human serum (10%) was used to prevent nonspecific binding of the second and third layer antibodies. A mouse IgG₁ myeloma protein was used as a negative control. Anti-CGRP monoclonal antibody was obtained from US Biological Inc./Europa Bioproducts Ltd (Wicken, UK) and is highly specific with no cross reaction with adrenomedullin with which it shares some sequence homology. The epitope for the anti-CGRP antibody has been defined as amino acids 28–37 on the human CGRP alpha chain (PheArgSerAlaLeuGluSerSer-ProAla) and this is not found anywhere in the adrenomedullin sequence (Dr John Peterson, USBiological Technical Support, personal communication). A polyclonal rabbit anti-CGRP was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Polyclonal rabbit anti-mouse immunoglobulin and APAAP reagents were purchased from Dakopatts. Double immunofluorescence staining was performed as previously reported (10).

For CGRP immunofluorescence tissue sections were pretreated with PBS for 30 min and incubated overnight at room temperature in a humidified chamber with the mouse antibody anti-CGRP. After extensive washing, sections were incubated with a FITC-conjugated goat anti-mouse Ab (Dako, Ely, UK) (11). Appropriate isotype

controls were included (IgG1, Dako). Images were acquired using a Leica TCS SP confocal microscope (Leica, Heidelberg, Germany).

NPs assay

Neuropeptides in the BAL supernatants were partially purified on C18 Sep-pak (Waters, Milford, MA, USA), eluted with acetonitrile: 1% trifluoro-acetic acid (60 : 40), dried and reconstituted in buffer prior to assay, as described previously (12). Neuropeptide recovery was >90%.

Neurokinin-A was measured using radioimmunoassay (RIA) using an *N*-terminus specific anti-serum (SK-570) which was raised in guinea pig to synthetic human NKA. The antibody cross-reacts with NKB and NPK (100%) not with SP (<0.1%). The detection limit for the assay is 2 ng/l.

Calcitonin gene-related peptide immunoreactivity was measured with a commercial CGRP human RIA kit (Peninsula Laboratories, Merseyside, UK) and the detection limit of the assay is 2 ng/l. This antibody is a rabbit anti-human CGRP peptide (II) antibody and it cross reacts with human CGRP (II), human CGRP, and rat CGRP (100%) but not with rat calcitonin C-terminal adjacent peptide (<0.001%) or with insulin, glucagon, somatostatin, SP, vasoactive intestinal peptide, and gastrin releasing peptide (<0.02%).

Substance P was measured with a commercially available ELISA (R&D Systems, Abingdon, UK). It shows no significant cross reactivity with NKA, NKB, and NPK. The limit of detection of this assay is 8 pg/ml.

BALF assays – protein correction factor

Bronchoalveolar lavage fluid samples were corrected for variable dilution using protein as an internal reference standard. Protein determination in BAL and concentrated BAL supernatants was performed using bicinchoninic acid protein assay kit (Sigma, St Louis, USA) according to the manufacturer's instructions.

The protein concentrations in all samples were normalized to the sample with the lowest protein concentration and all values obtained in the assays were corrected accordingly.

Statistical analyses

Data from BALF supernatant assays, BAL cytopins, BB immunohistochemistry, and *in situ* hybridization analyses are expressed as medians, with minimum to maximum ranges shown. Statistical comparisons of diluent and peptides inhalation (in-group comparisons) were performed using two-tailed Wilcoxon signed-rank tests. Between-group comparisons of responders and nonresponders were performed using the nonparametric Mann-Whitney test. Correlations with clinical characteristics were performed using Spearman's rank coefficient of correlation.

Results

Clinical features

As previously described (6) the 12 responders and 12 nonresponders were well matched at baseline for gender (ratio male : female 4 : 8 for responders vs 6 : 6 for nonresponders), age [median 27 years (range 26–34 years) responders vs 28 years (range 23–29 years) nonresponders], FEV₁ [median and range 93.8% predic-

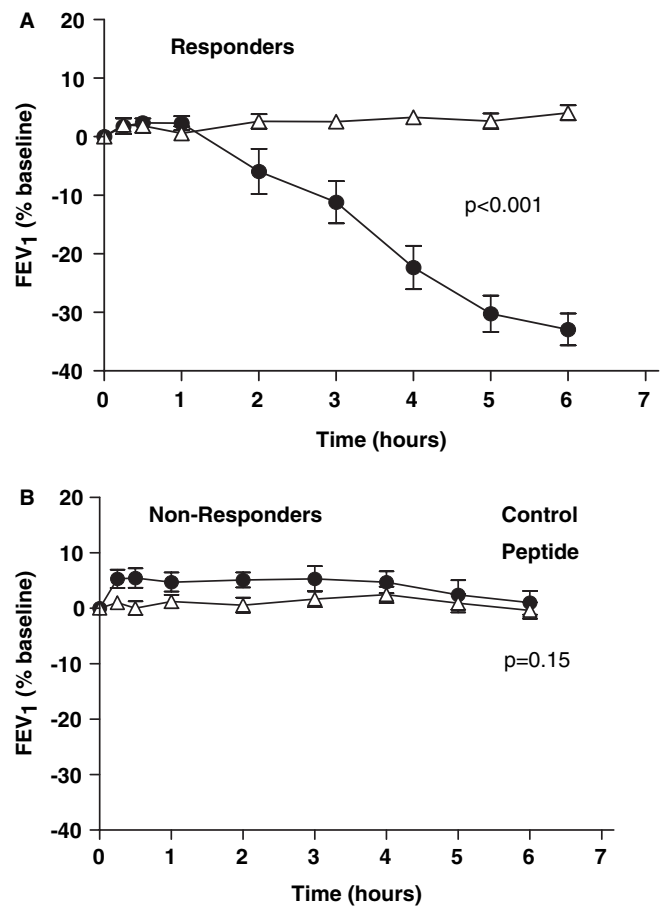


Figure 1. Characteristics of T-cell peptide responders and nonresponders. The changes in FEV₁ after peptide (closed circles) challenge in 12 responders (A) and 12 nonresponders (B) is shown, as well as the effect of diluent (open triangles). The spirometry data is also documented elsewhere (6).

ted (87.5–102.7) responders vs 92.8 (86.1–95.6) nonresponders and histamine PC₂₀ (3.99 mg/ml (1.67–12.5) responders vs 3.99 (1.93–4.63) nonresponders]. The cat radioallergosorbent test (IU/ml) was significantly higher in responders [29.2 IU/ml (range 6.17–43.9)] than nonresponders (3.49 IU/ml (range 1.98–7.07)) in nonresponders ($P = 0.002$). The changes in FEV₁, following inhalation of either peptide or diluent, in responders and nonresponders, are shown in Fig. 1A and B, respectively (and in reference 6). On the peptide day responders had an average decrease in FEV₁ at 6 h of approximately 33% ($P < 0.001$). There was virtually no change, between baseline and 6-h FEV₁ values, on the diluent day in responders, or on diluent or peptide days in nonresponders.

Measurements in BAL

The concentrations of NPs in BAL are shown in Fig. 1. There were no significant between group differences

(responders vs nonresponders, diluent vs peptide) in the NPs, NKA, or SP. With NKA there was a within group increase in responders ($P = 0.05$) but not nonresponders. In contrast there was a highly significant difference in BAL between the groups in the concentration of CGRP ($P = 0.009$, Fig. 1). Furthermore, a within group significant increase was only observed for responders ($P = 0.04$) but not nonresponders.

CGRP Immunoreactivity

Calcitonin gene-related peptide immunoreactivity in the epithelium, below the basement membrane and in smooth muscle is shown in Figs 3 and 4. There were marked increases in CGRP+ cells (per mm) within the epithelium (between group difference $P = 0.0005$) (Figs 3A and 4A–D), below the basement membrane (between group difference $P = 0.0005$) (Figs 3B and 4E,F) and in association with bronchial smooth muscle (between group difference $P = 0.02$) (Fig 3C,E and F) in responders only. Shedding of the epithelium was observed in both responders and nonresponders on both the diluent and peptide days. Thus, in responders, after peptide provocation CGRP+ cells were observed in both intact and disrupted epithelium (Fig. 4A–D). Anti-CGRP appeared to recognize the protein in most epithelial cells, i.e. staining was not clustered or confined to one epithelial cell type.

Figure 4G shows a confocal microscope image of CGRP+ immunofluorescence on epithelial cells and smooth muscle. A control (Fig. 4H) showed virtually no fluorescence. Increases in the numbers of CGRP+ cells within the epithelium and below the basement membrane were also associated with diffuse extracellular (presumed sensory fiber) staining but this was not formally quantified.

In responders, postpeptide challenge, the inflammatory cells in the lamina propria which expressed CGRP were mainly CD3+, CD4+, and CD68+ macrophages. Thus, by double staining it was shown that CGRP co-localized mainly to CD3+, CD4+, and to a lesser extent to CD68+ cells with very CGRP staining by CD8+ cells, neutrophils, or eosinophils (Fig. 5A). Thus in seven of the peptide responder biopsies studied between 60% and 100% of the CD3+ cells were CGRP positive whereas about 10–50% of CD4+ and a variable percentages of macrophages stained with anti-CGRP (Fig. 5B). Fig. 6 shows examples of CGRP co-staining with CD3+ cells and CD68+ cells.

Discussion

The provocation of isolated LAR by inhalation of allergen-derived T-cell epitopes represents a unique model for studying asthmatic mechanistic events which result from initial activation of T-cells, independent of

the contribution from mast cells and other IgE-bearing cells.

In a previous study we examined BB and BALF from responders and nonresponders 6 h after either intradermal injection of allergen-derived peptides or diluent control (4). Surprisingly, we found that peptide challenge was not associated with changes in numbers of eosinophils, neutrophils, basophils, mast cells, T-cell subsets, macrophages, Th2 cytokines, histamine, histamine-releasing factors, neurotrophins, or eicosanoids in airway samples, i.e. BAL or biopsies. However, as the challenge route was systemic (intradermal), 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 challenge method previously reported (3). A similar study group of responders and nonresponders was obtained (Fig. 1). In a separate report studying BALF from these same patients we confirmed that, like the intradermal route, peptide-induced LARs provoked by the inhaled route were not associated with increases in eosinophils, neutrophils, or basophils or a range of pharmacological mediators including histamine, eicosanoids or the complement fragment C3a or C5a (6).

In the present study we have focused on NPs as these were not measured in our previous report using the intradermal route for challenge (4). Only CGRP concentrations but not SP or NKA were significantly elevated in BAL when responders were compared with nonresponders (Fig. 2). The within group responder values only just reached significance ($P = 0.04$) and the highly significant between group value ($P = 0.009$) may have been a reflection of the slight decrease in nonresponders after peptide challenge. Nevertheless, it encouraged us to study CGRP expression in biopsy material obtained at the same time as BAL. Thus, we were able to show that CGRP immunoreactivity was markedly upregulated at several levels of the airway wall in virtually all responders, but not nonresponders (Fig. 3). These novel findings raise the possibility that LARs have an appreciable vascular component and that the CGRP may be a major mediator facilitating vasodilatation and subsequent edema in the asthma process (although further studies are required to establish this).

Calcitonin gene-related peptide is a 37 amino acid peptide resulting from alternative splicing of mRNA from the calcitonin gene (13). It is part of the adrenomedullin, calcitonin, amylin family of polypeptides and a potent arterial and venous vasodilator (14). Brain *et al.* (15) showed, in experimental animals and man, that CGRP had a prolonged mode of action when injected into the skin (5–6 h with as little as 15 pmols). Calcitonin gene-related peptide does not induce permeability *per se* but, appears to act synergistically with several mediators of inflammation, including histamine, to produce marked and prolonged edema (16). In the airways, in health, CGRP is contained within small

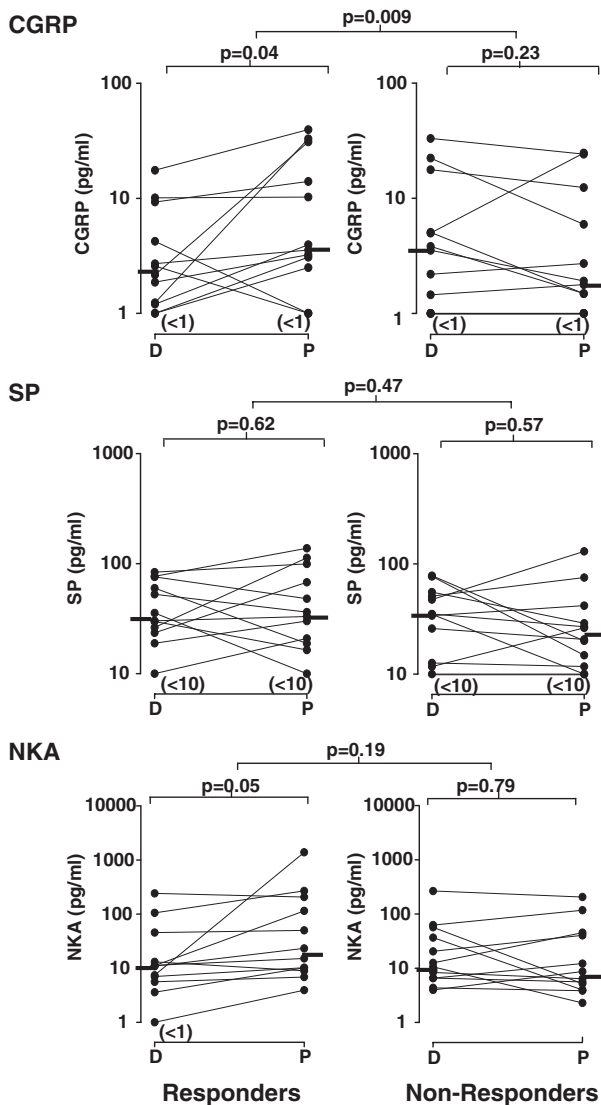


Figure 2. Concentrations of calcitonin gene-related peptide, substance P and neurokinin A in bronchoalveolar lavage fluid from peptide responders and nonresponders.

sensory nerves, epithelial neuroendocrine cells (NEC) and aggregates of NEC, neuroendocrine bodies. However, our findings indicate that at the height of the LAR there is a diffuse pattern of staining of cells within the epithelium and in inflammatory cells below the basement membrane, an observation in keeping with those in experimental animals. Thus, Aoki-Nagase *et al.* (17) showed CGRP immunoreactivity throughout the airway epithelium and in the submucosa of mice sensitized and challenged with specific antigen. This was similar in distribution to that observed in biopsies from our peptide-induced LAR (Figs 3 and 4). Furthermore, sensitized, CGRP gene-disrupted, mice had significant attenuation of both airway hyperreactivity (AHR) and CGRP expression after antigen challenge. Calcitonin

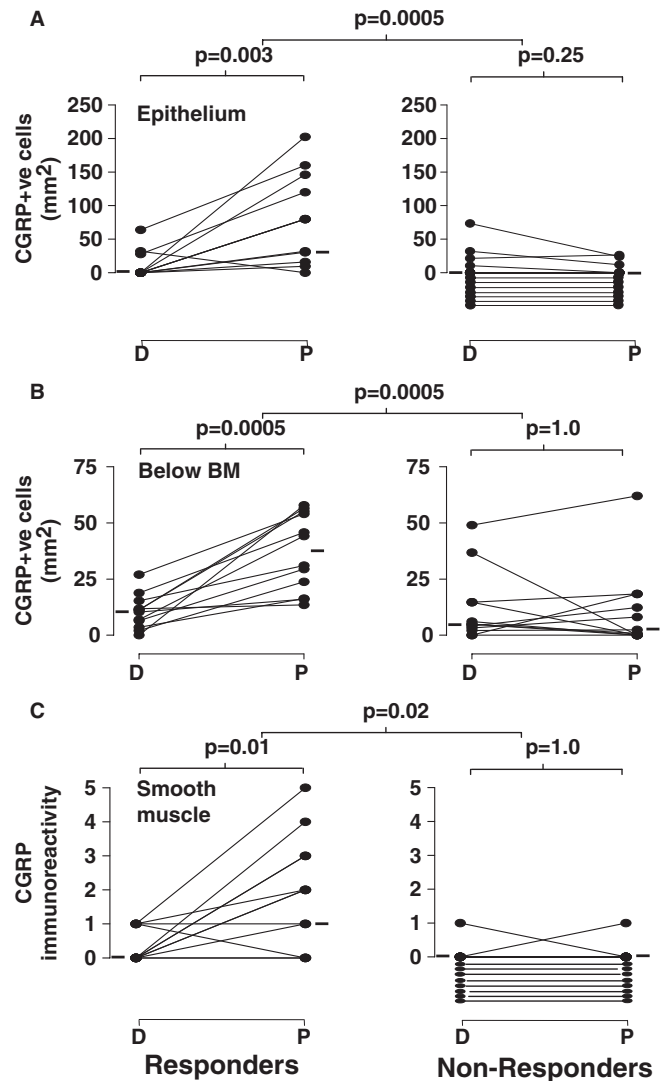


Figure 3. Calcitonin gene-related peptide (CGRP+) cells in the airway wall of responders and nonresponders. The numbers of CGRP+ cells in the epithelium is shown in A and the numbers of immunoreactive infiltrating inflammatory cells below the basement membrane is shown in B. Smooth muscle CGRP immunoreactivity graded on an arbitrary scale 0–5 is shown in figure C. For clarity all the zero values or nonresponders in panel A and C have been depicted.

gene-related peptide has also been shown to be synthesized and secreted *in vitro* by the type II alveolar cell line, A549 (18). There are also many studies showing that the related NP, SP, is expressed by several inflammatory cells, including neutrophils and eosinophils (19). There is controversy regarding the effects of CGRP on bronchial smooth muscle. Earlier claims that it is a potent bronchoconstrictor (20) have not been confirmed and in any event, this would have been surprising as CGRP increases levels of cyclic adenosine monophosphate in airway smooth muscle cells indicating

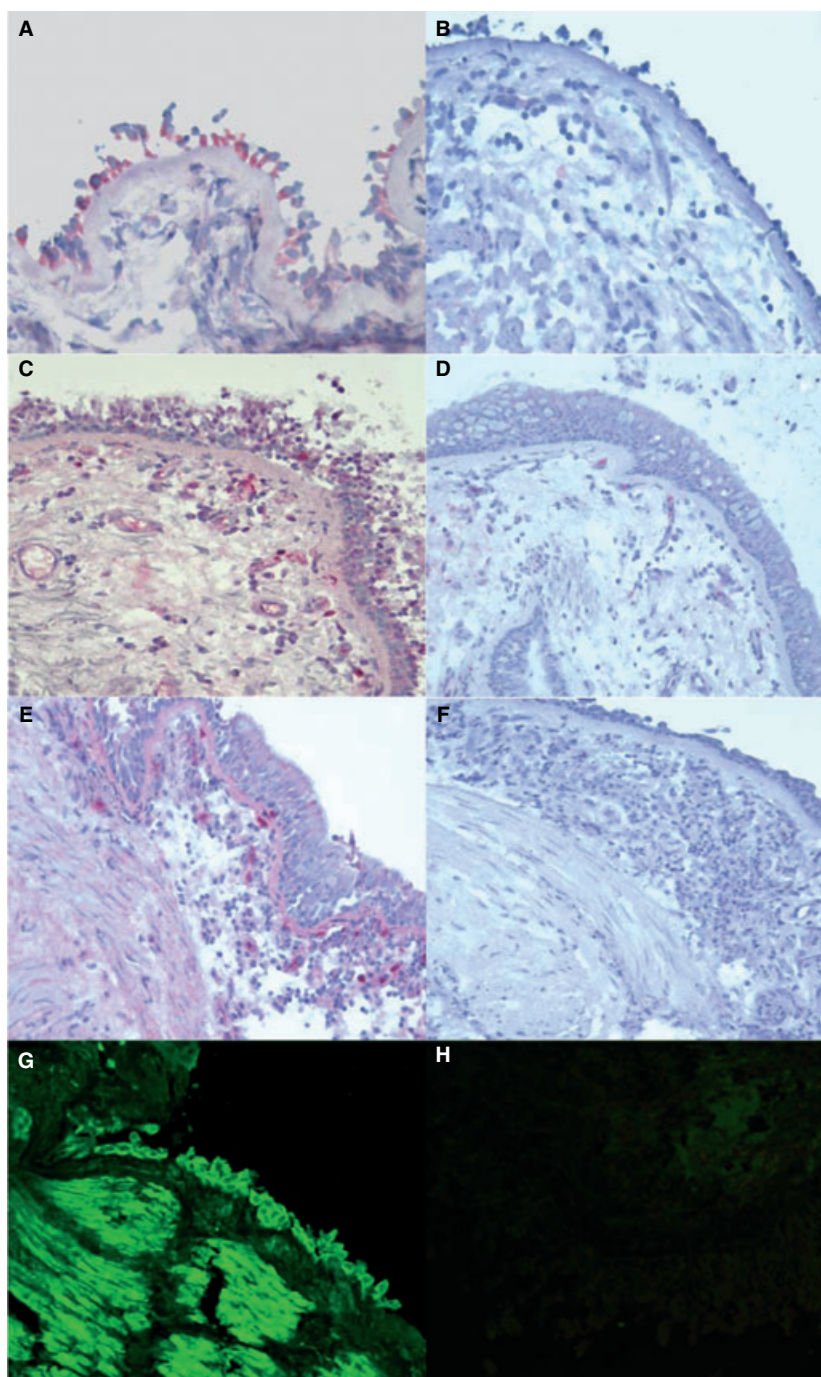


Figure 4. Photomicrographs of calcitonin gene-related peptide (CGRP) immunoreactivity in mucosal bronchial biopsies from peptide-induced late asthmatic reactions. (A) Responder challenged with peptides showing CGRP immunoreactive cells in basal cells along a length of basement membrane. The epithelium is denuded. (B) Responder challenged with diluent showing basal cells and denuded epithelium but no immunoreactivity. (C) Responder challenged with peptide showing numerous immunoreactive cells within a disrupted epithelium together with positive infiltrating cells below the basement membrane as well as in association with small blood vessels. (D) A nonresponder after peptide challenge. There are occasional CGRP immunoreactive cells below the basement membrane. The epithelium is intact. (E) Responder challenged with peptide showing numerous infiltrating CGRP+ inflammatory cells below the basement membrane and also immunoreactivity in association with airway smooth muscle. (F) A responder challenged with diluent showing no immunoreactive submucosal inflammatory cells or CGRP staining in association with airway smooth muscle. (G) A confocal micrograph of a responder challenged with peptide showing CGRP+ epithelial cells and immunoreactive smooth muscle. (H) A nonresponder challenged with peptide showing no smooth muscle or epithelial immunoreactivity.

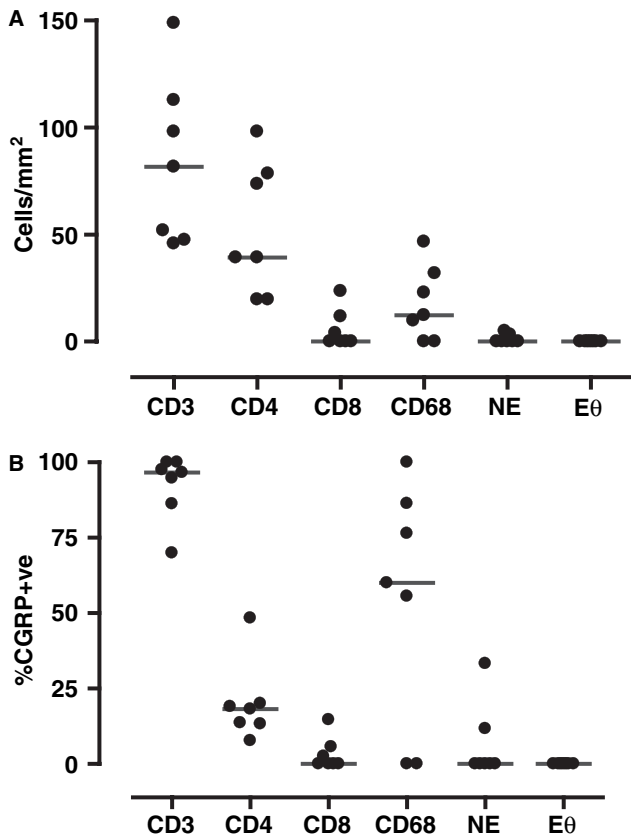


Figure 5. Co-localization of calcitonin gene-related peptide (CGRP) immunoreactivity to CD3+ and CD4+ T lymphocytes, neutrophils (NE), eosinophils (EO) and CD68+ macrophages. The numbers of double positive cells below the basement membrane is shown in A. the percentage of the various cell types which are CGRP positive is shown in B.

a relaxing effect. On the other hand, the peptide has been shown to have constrictor effects on damaged (epithelium-denuded) human airways (21). To date there

is no convincing evidence that CGRP is overtly expressed in asthma or allergic disease although previous studies were performed on biopsies from asthmatics obtained at baseline (22,23). Dakhama *et al.* (24) suggested that CGRP may have a regulatory role as its administration to sensitized and challenged mice resulted in the normalization of AHR. Nevertheless our present clinical data indicates that changes in the vasculature may be critical in the pathogenesis of late-phase allergic reactions and, if confirmed, will have important implication for understanding mechanisms in bronchial, nasal, and cutaneous late-phase allergic reactions.

Calcitonin gene-related peptide immunoreactivity was demonstrable not only in the epithelium and in association with airway smooth muscle but also in inflammatory cells infiltrating the submucosa (Figs 3 and 4C,E). Co-localization experiments with seven of the subjects showed that these were largely CD3+/CD4+ and CD68+ cells (Fig. 5). Further studies are required to account for the CD3+/CGRP+ cells which were apparently not CD4+ or CD8+. This could either be a technical issue relating to different affinities of the reagent used or possibly a reflection of a CD3+/CD4-/CD8- NKT cell population recently associated with asthma immunopathology (although we have not tested for this because of limitations of biopsy samples) (25,26). In previous studies we had difficulty identifying protein mediators in T-cells by immunostaining using the APA-AP technique (9). However the present study employs double immunofluorescence and confocal microscopy and is presumably more sensitive at detecting cytoplasmic proteins/peptides (10).

In any event our work supports the findings of Wang *et al.* who showed production and secretion of CGRP from human lymphocytes in vitro (27). The concept that T-cells and macrophages can be recruited in allergic airway disease for the release of the vasoactive peptide

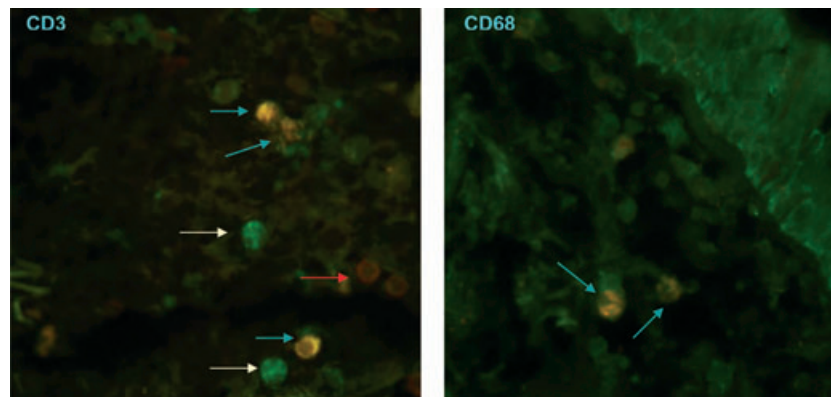


Figure 6. Photomicrograph of confocal microscopy. The upper panel shows co-localization of calcitonin gene-related peptide (CGRP) to CD3 cells (orange - indicated by blue arrows). Single CGRP cells are green (indicated by white arrows) and single CD3+ cells are red (indicated by red arrows). Double positive (yellow) CGRP/CD68+ cells are shown in the lower panel.

CGRP is novel and supports the view that vascular leakage may be a component of subacute (i.e. LAR) as well as acute airway narrowing.

We do not believe, however, that enhanced CGRP expression in late-phase allergic reactions is solely confined to our peptide challenge asthma model. Preliminary data in six subjects also shows that CGRP is markedly, but transiently, expressed by inflammatory cells during late-phase skin responses in atopics challenged intradermally with whole allergen extract (Kay AB, Benyahia F, Barkans J, unpublished observations). The CGRP⁺ cells decreased in parallel with the regression of the late-phase reaction whereas CGRP⁻ inflammatory cells appeared to persist at the site of injection.

In conclusion our present results indicate that in our model of human asthma CGRP is markedly expressed at all levels of the airway wall including epithelial cells, infiltrating inflammatory cells and in association with smooth muscle. These observations are in keeping with a number of studies on the role of the vasculature in asthma. These include the demonstration of increases in angiogenic factors such as vascular endothelial growth factor and angiogenin (28) as well as stromal cell-derived

factor 1 in asthmatic airway epithelium (29). Furthermore, increases in subepithelial vessels of the airways were observed in newly diagnosed asthmatics, but not in chronic obstructive pulmonary disease patients or normal controls using a high magnification broncho-videoscope (30).

Taken together, these studies, and our present findings, indicate that vascular permeability and edema of the airways may make an important contribution to airway narrowing in LAR or even ongoing asthma, and that CGRP, may be a critical mediator in these events. However, further studies are required before firm conclusions can be drawn regarding the functional significance of our finding of elevated CGRP in this model of chronic asthma in man.

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