

Interleukin-4 and -13 expression is co-localized to mast cells within the airway smooth muscle in asthma

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Abstract

Background Airway smooth muscle infiltration by mast cells is a feature of asthma and not eosinophilic bronchitis. In asthma, Th2 cytokines have been implicated as playing a critical role in the development of airway inflammation and hyper-responsiveness. Whether inflammatory cells within the airway smooth muscle release these cytokines is unknown.

Methods We have undertaken a comparative immunohistochemical study in bronchial biopsies from 14 subjects with asthma, 10 with eosinophilic bronchitis and eight normal controls recruited from two centres.

Results The median number of IL-4⁺ cells/mm² smooth muscle was significantly higher in subjects with asthma than eosinophilic bronchitis and normal controls for both the anti-IL-4 mAb 3H4 (2.4, 0, 0, respectively; $P = 0.001$) and anti-IL-4 mAb 4D9 (1.6, 0, 0, respectively; $P = 0.02$). There were no group differences in the number of IL-5⁺ cells ($P = 0.31$). In six subjects with asthma, IL-13 expression by cells within the airway smooth muscle was studied. The median (range) of IL-13⁺ cells was 2 (0.9–2.7). Ninety-four percent of the cells expressing IL-4 (3H4), 92% of those expressing IL-4 (4D9) and 100% expressing IL-13 in the airway smooth muscle were mast cells. Fifty-five percent of the mast cells within the airway smooth muscle co-localized to IL-4 (3H4), 29% to IL-4 (4D9) and 17% to IL-13.

Conclusions In asthma, IL-4⁺ and IL-13⁺ cells were present within the airway smooth muscle and were expressed predominantly by mast cells, suggesting that IL-4 and IL-13 may play an important role in mast cell–airway smooth muscle interactions.

Keywords airway hyper-responsiveness, airway smooth muscle, asthma, eosinophilic bronchitis, eosinophils, IL-4, IL-5, IL-13, mast cells, Th2 cytokines, T lymphocytes

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Introduction

Asthma is a condition characterized by variable airflow obstruction and airway hyper-responsiveness in association with airway inflammation. Many of the pathological features of asthma are well-established such as accumulation of eosinophils and CD4⁺ lymphocytes, mast cell degranulation, mucus hypersecretion, thickening of the sub-epithelial collagen layer, and smooth muscle hypertrophy and hyperplasia [1].

In addition to these traditional features, we have recently reported that mast cells infiltrate the airway smooth muscle in asthma, but not in eosinophilic bronchitis or normal controls [2]. This observation is of particular interest as eosinophilic bronchitis, a condition characterized by corticosteroid responsive cough [3, 4], shares other immunopathological

features of asthma. For example, there is active eosinophilic airway inflammation with release of important effector mediators [2, 5], Th2 cytokine expression [6, 7] and reticular lamina sub-basement membrane thickening, but no physiological abnormality in terms of variable airflow obstruction or airway hyper-responsiveness. Thus, microlocalization of mast cells within the airway smooth muscle bundle may be a key factor in the development of the disordered airway physiology observed in asthma.

There is increasing evidence that the development and maintenance of airway inflammation in asthma is due to the production of Th2 cytokines such as IL-4, IL-5 and IL-13 [8]. In addition to T lymphocytes, mast cells are an important source of these cytokines [9]. Airway smooth muscle cells express receptors for IL-4, IL-5 and IL-13 [10, 11], and interestingly, *in vitro* at least, IL-5 [11] and IL-13 [10] induce airway hyper-responsiveness by directly activating airway smooth muscle cells. We therefore hypothesized that inflammatory cells within the airway smooth muscle bundles express Th2 cytokines.

To test our hypothesis, we have undertaken a comparative immunohistochemical study using mAb to IL-4, IL-5 and IL-13

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in bronchial mucosal biopsies obtained from symptomatic asthmatic subjects, patients with eosinophilic bronchitis and normal controls.

Methods

Subjects

Subjects were recruited from Leicester and Southampton. They had participated in a previously published study investigating the cellular infiltration of airway smooth muscle in asthma [2]. Fourteen subjects with asthma, 10 with eosinophilic bronchitis and eight normal controls were recruited. The subject characteristics are shown in Table 1.

Subjects with asthma gave a suggestive history and had objective evidence of variable airflow obstruction as indicated by one or more of the following: (1) methacholine airway hyper-responsiveness (PC_{20} forced expiratory volume in 1 s (FEV_1) < 8 mg/mL); (2) > 15% improvement in FEV_1 10 min after 200 µg inhaled salbutamol; or (3) peak expiratory flow (PEF) (> 20% maximum within day amplitude from twice daily PEF measurements over 14 days). The subjects with eosinophilic bronchitis had a persistent isolated cough, no symptoms suggesting variable airflow obstruction while under regular review for at least 2 months, normal PEF variability (maximum within day amplitude % mean < 20% over 2 weeks), no bronchodilator reversibility, a normal chest radiograph and, on at least two occasions separated by more than 2 months, normal spirometric values, a methacholine PC_{20} > 16 mg/mL and a sputum eosinophilia (> 3% non-squamous cell). Normal subjects were asymptomatic and had no evidence of variable airflow obstruction or airway hyper-responsiveness. All subjects were current non-smokers with a past smoking history of less than 10 pack years. None of the subjects had taken inhaled or oral corticosteroids for at least 6 weeks prior to the study and managed their asthma with short-acting β_2 -agonists taken as required. The study was approved by the Leicestershire and Southampton Ethics Committee and all patients gave their written informed consent.

Protocol and clinical measurements

Subjects attended on two occasions. At the first visit they had spirometry, allergen skin prick tests, a methacholine inhalation test followed on recovery by sputum induction in those

subjects at the Leicester site. Spirometry was performed using a dry bellows spirometer (Vitalograph, Buckingham, UK) with the FEV_1 recorded as the best of successive readings within 100 mL. Allergen skin prick tests were performed to *Dermatophagoides pteronyssinus*, cat fur, grass pollen and *Aspergillus fumigatus* solutions with normal saline and histamine controls (Bencard, Nottinghamshire, UK). A positive response to an allergen on the skin prick tests was recorded in the presence of a weal > 2 mm more than the negative control. The methacholine challenge was performed using the tidal breathing method [12] with doubling concentrations of methacholine (0.03–128 mg/mL) nebulized via a Wright nebulizer. We extended the dose range to include high doses in order to explore fully the dose–response relationship. Sputum was induced and processed as previously described [13].

At the second visit, bronchial mucosal biopsies were taken under local anaesthesia from the right middle and lower lobe carinae via an Olympus fibre-optic bronchoscope (Olympus Company, Tokyo, Japan) in line with the most recent British Thoracic Society guidelines [14]. All subjects received nebulized 2.5 mg salbutamol. Lignocaine (1–4%) was used for local anaesthesia and continuous oxygen given via nasal cannulae throughout the procedure. Mucosal biopsies were immediately transferred into ice-cooled acetone containing the protease inhibitors iodoacetamide (20 mM) and phenylmethyl-sulfonylfluoride (PMSF) (2 mM) for fixation, stored at -20°C for 24 h, and then processed into the water-soluble resin glycolmethacrylate (GMA) (Polysciences, Northampton, UK) for embedding.

Immunohistochemistry

The technique of immunostaining applied to GMA embedded tissue has been described previously [15]. Two-micrometre sections were cut, floated on 0.2% ammonia solution in water for 1 min and dried at room temperature for 1–4 h. The following mouse monoclonal antibodies were used: IL-4 (3H4) (AMS Biotechnology, Abingdon, UK), IL-4 (4D9) (AMS Biotechnology), IL-5 (a gift from GlaxoSmithKline, Harlow, UK) and IL-13 (R&D Systems, Abingdon, UK). Sequential sections were stained for mast cell tryptase (AA1, Dako Ltd, High Wycombe, UK) to assess the co-localization of these cytokines with mast cells. Appropriate control sections were similarly treated either with the primary mAb omitted or in the presence of an unrelated antibody of the same isotype (Dako Ltd).

Assessment and quantification of immunohistochemical staining

Areas of airway smooth muscle were identified morphologically and the area was calculated using a computer analysis system (Scion Image, Scion Corporation, Maryland, UK). We have previously validated this technique [2]. Nucleated immunostained cells present in coded sections were enumerated in the airway smooth muscle, and numbers of cells were expressed as the number/mm² of smooth muscle. Cells were counted within the smooth muscle bundles but not adjacent to them, and were confirmed to be in the substance of the smooth muscle by serial sections to avoid counting cells

Table 1. Subjects' clinical characteristics

	Eosinophilic bronchitis	Asthma	Normal
Number	10	14	8
Age*	47 (13)	39 (15)	35 (20)
Male	6	8	6
Atopy	5	11	2
PC_{20} FEV_1 †	94 (56–128)	0.4 (0.03–4.6)	128 (16–128)
FEV_1 % predicted*	101 (11)	102 (15)	97 (16)

*Mean (SD). †Median (range).

within the mucosal tissue that were juxtaposed due to biopsy artefact. Mast cells and cells positively expressing cytokines within the airway smooth muscle were co-localized using the image analysis system.

A minimum area of 0.1 mm² from a biopsy section of airway smooth muscle was considered to be sufficient to assess cellular infiltration, and two to four sections at least 10 µm apart were assessed for each patient from one to two biopsies. The area of smooth muscle for each subject was expressed as the mean of all the assessable sections. All counts were performed blind to clinical characteristics, and the specimens from the three groups were intermingled during processing and counting. Additional sections were obtained from the biopsies of six subjects with asthma who had assessable airway smooth muscle. The expression of IL-13 by cells within the airway smooth muscle bundles was only assessed in these subjects.

Statistical analysis

Subject characteristics were described using descriptive statistics. Cell counts were expressed as medians (ranges). Comparisons across the three groups were undertaken using the Kruskal–Wallis test and the Mann–Whitney *U*-test was used to compare between groups. A value of $P < 0.05$ was taken as being statistically significant.

Results

The median (range) area (mm²) of smooth muscle assessed per biopsy section was similar between the subjects with asthma 0.3 (0.16–0.97), eosinophilic bronchitis 0.35 (0.1–1.9) and normal controls 0.3 (0.12–0.91) ($P = 0.61$).

Staining for the mAb 4D9 was cytoplasmic whereas for mAb (3H4) produced peripheral ring staining. Examples of sequential sections with positive cellular staining for IL-4 (3H4), IL-4 (4D9) and IL-13 co-localized with tryptase⁺ cells in the airway smooth muscle are shown (Figs 1–3). The individual values for the number of cells expressing IL-4 (3H4, 4D9) in the airway smooth muscle are as shown (Fig. 4). The median (range) IL-4 (3H4)⁺ cell count/mm² was significantly higher in the subjects with asthma 2.4 (0–9.5) than in eosinophilic bronchitis 0 (0–1.4) (95% confidence interval (CI) of difference 0.8, 3; $P = 0.003$) or normal controls 0 (0–1) (95% CI of difference 1.1, 3.3, 2; $P = 0.002$). The median (range) IL-4 (4D9)⁺ cell count/mm² was significantly higher in the subjects with asthma 1.6 (0–7.1) than in eosinophilic bronchitis 0 (0–1.7) (95% CI of difference 0, 2.8; $P = 0.02$) or normal controls 0 (0–1) (95% CI of difference 0, 2.9; $P = 0.03$).

There were no group differences in the median (range) cells/mm² smooth muscle IL-5⁺ cells in those subjects with asthma 0 (0–1.7), eosinophilic bronchitis 0 (0–1.4) and normal controls (0–0.3) ($P = 0.31$). Counts in atopic and non-atopic subjects within groups were similar.

In six subjects with asthma, IL-13 expression was studied and the median (range) number of IL-13⁺ cells was 2 (0.9–2.7). IL-4, IL-5 and IL-13 were not expressed by the airway smooth muscle cells.

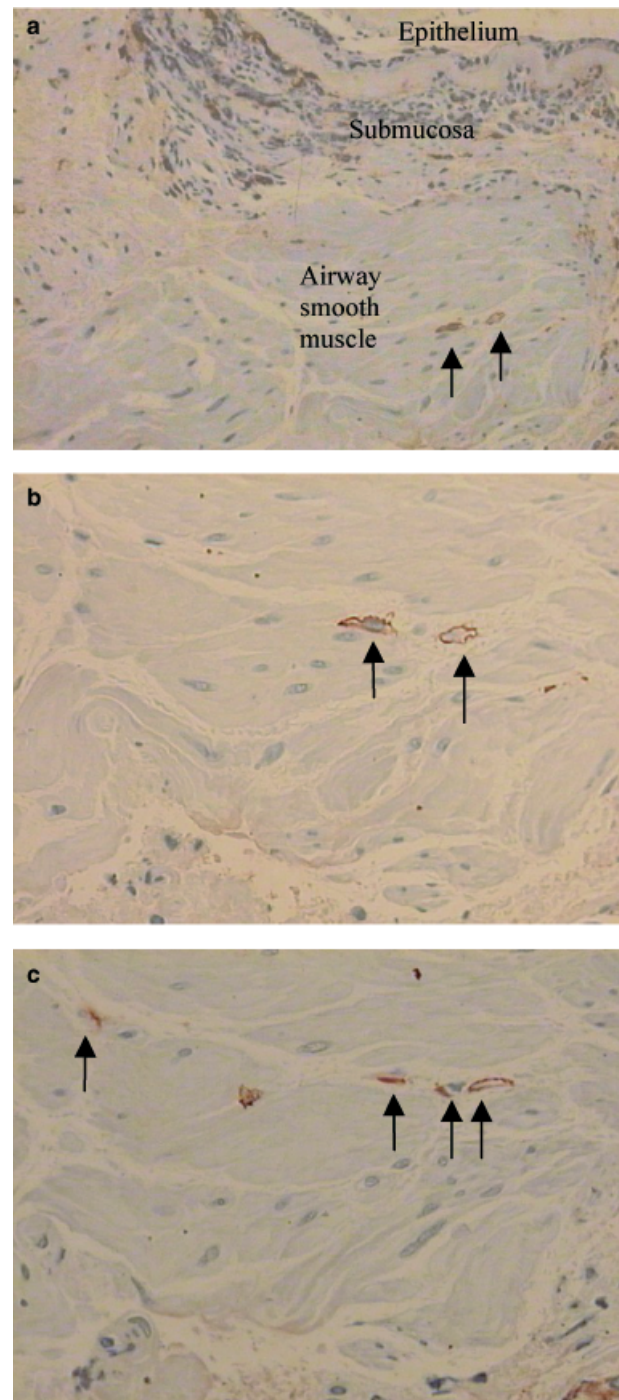


Fig. 1. Bronchial biopsies from subjects with asthma (a) demonstrating epithelium, submucosa and smooth muscle with cells expressing IL-4 (3H4) (see arrows) infiltrating the airway smooth muscle ($\times 100$); (b and c) sequential sections of IL-4 (3H4) and tryptase positive cells, respectively, in the airway smooth muscle ($\times 200$).

Comparison of sequential sections in the subjects with asthma demonstrated that 94% of the cells expressing IL-4 (3H4), 92% of those expressing IL-4 (4D9) and 100% expressing IL-13 in the airway smooth muscle were mast cells. Fifty-five percent of the mast cells within the airway smooth muscle co-localized to IL-4 (3H4), 29% to IL-4 (4D9) and 17% to IL-13.

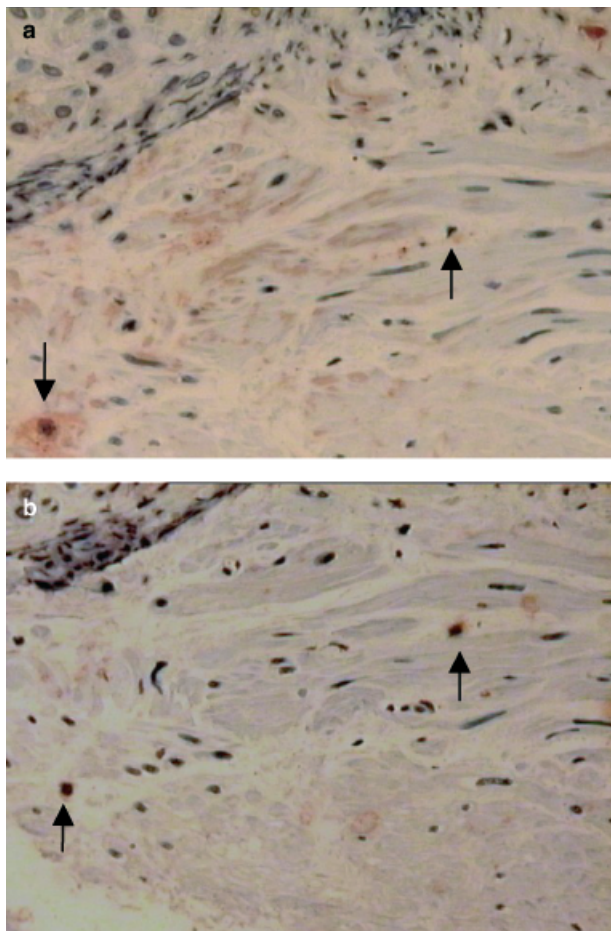


Fig. 2. (a and b) Sequential sections of IL-4 (4D9) and tryptase positive cells, respectively, in the airway smooth muscle (see arrows, $\times 200$).

Discussion

This study has, for the first time, described the expression of the Th2 cytokines IL-4, IL-5 and IL-13 by inflammatory cells within the airway smooth muscle bundles in patients with asthma. We have demonstrated that inflammatory cells within the airway smooth muscle, which were predominantly mast cells, commonly express IL-4 and IL-13 but not IL-5. This observation provides evidence that in asthma, mast-cell-derived IL-4 and IL-13 secreted locally within the smooth muscle bundles may play an important role in the disordered airway smooth muscle physiology that characterizes this disease.

Mast cells localized within the bronchial submucosa express a number of cytokines including IL-4, IL-5, IL-6 and TNF- α [9], and store IL-4 within the matrix of their granules [16]. In addition, we have recently reported that IL-4 expression is increased and co-localized to mast cells in the bronchial submucosa in eosinophilic bronchitis [7]. In this current study, we found that airway smooth muscle mast cells expressed IL-4 and IL-13, but in contrast IL-5 immunoreactive cells were rarely observed within the airway smooth muscle. It has been suggested that the cytoplasmic staining of mAb IL-4 (4D9) represents stored IL-4, whereas mAb 3H4 is a marker of released IL-4 expressed on the surface of the mast

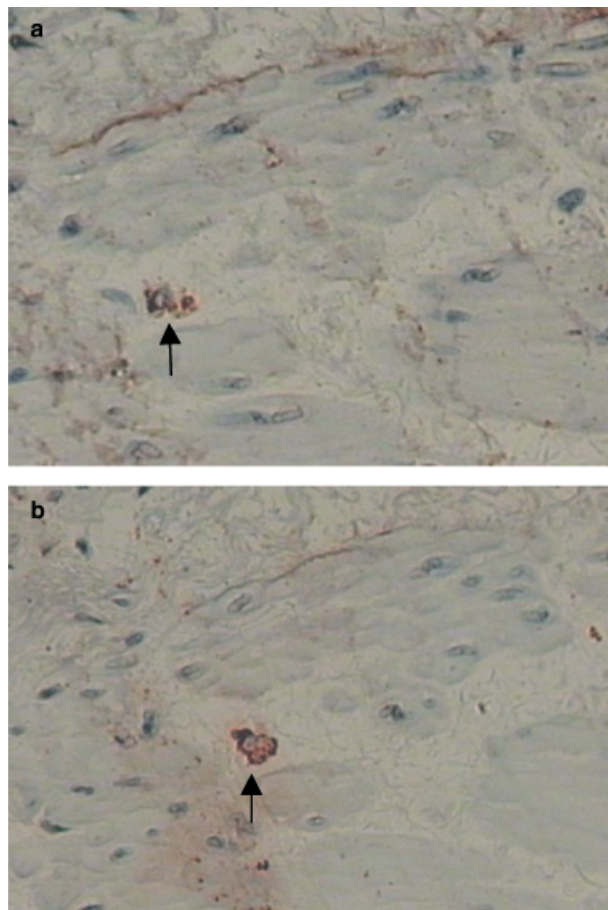


Fig. 3. (a and b) Sequential sections of IL-13 and tryptase positive cells, respectively, in the airway smooth muscle (see arrows, $\times 400$).

cells [9]. In this study, the number of cells that expressed IL-4 with either IL-4 mAb was broadly similar. Mast cells can be classified by their constituent proteases into tryptase and chymase positive mast cells (MC_{TC}) or tryptase alone (MC_T). In subjects with asthma, allergic rhinitis [17] and allergic conjunctivitis [18], IL-4 and IL-13 co-localize preferentially within MC_{TC} mast cells, whereas IL-5 and IL-6 localize to the MC_T subset. We found that mast cells within the airway smooth muscle bundles were also predominantly MC_{TC} [2], which provides a likely explanation for the absence of IL-5. This evidence suggests that differences in protease phenotype may also reflect functional differences evidenced by the different patterns of cytokine distribution.

In asthma, IL-4 and IL-13 have established and diverse roles in the development of airway mucosal inflammation [19]. There is also emerging evidence that these cytokines exert important biological effects on human airway smooth muscle. Airway smooth muscle cells express the IL-4 high-affinity α chain of the IL-4 receptor (IL-4R α) and the IL-13R α I and IL-13R α II chains, but not the common γ chain. IL-4 and IL-13 are known to trigger airway smooth muscle cell signalling via the IL-4R through activation of both signal transducer and activator of transcription (STAT)-6 and extracellular signal-regulated kinase (ERK) [10]. IL-13, but not IL-4, has been shown to attenuate relaxation to β -agonists

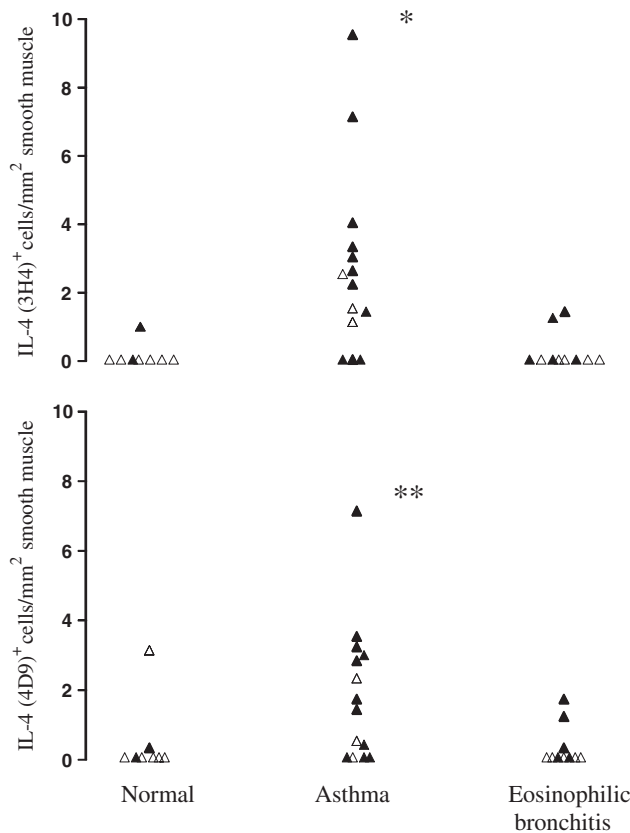


Fig. 4. Cell counts/mm² airway smooth muscle for cells expressing IL-4 (4D9) and IL-4 (3H4). * $P = 0.001$ (Kruskal–Wallis test), ** $P = 0.02$ (Kruskal–Wallis test). Closed triangles, atopic subjects; open triangles, non-atopic subjects.

and augment contractility to acetylcholine, suggesting that IL-13 may play an important role in the development of airway hyper-responsiveness [10, 20]. Interestingly, IL-4 and IL-13 have opposing effects on airway smooth muscle proliferation. IL-13 up-regulates the cysteinyl-leukotriene D4 receptor, which following activation by cysteinyl-leukotriene D4 leads to airway smooth muscle proliferation [21]. In contrast, IL-4 has been shown to inhibit proliferation [22]. In addition to affecting airway smooth muscle proliferation and contractile responses, IL-4 and IL-13 have been reported to increase the secretion of chemokines such as eotaxin and TARC by airway smooth muscle cells [23–25]. There is recent evidence that would support the hypothesis that the release of eotaxin by airway smooth muscle cells may result in a positive feedback mechanism whereby further mast cells are attracted into the airway smooth muscle. Eotaxin is a chemokine for mast cells acting via the chemokine receptor CCR3 [26]. CCR3 is expressed by only 20% of lung mast cells, but these are almost exclusively MC_{TC} [26, 27], which is the predominant mast cell phenotype in the airway smooth muscle.

The importance of IL-4 in the development of airway inflammation in asthma is supported by the results of the early clinical trials, which have demonstrated that human recombinant (rhu) IL-4 receptor (R) treatment is safe and effective [28, 29]. In these reports, subjects received nebulized rhuIL-4R or placebo weekly over a 3-month period following inhaled corticosteroid withdrawal. In those subjects treated

with rhuIL-4R, there was improvement in the asthma quality of life score and nitric oxide with no change in FEV₁ compared with worsening symptoms and lung function in the placebo group. In contrast, anti-IL-5 treatment markedly reduces the sputum eosinophil count but does not affect airway responsiveness or the physiological response to inhaled allergen [30]. The microlocalization of mast cells expressing IL-4, but not IL-5, within airway smooth muscle therefore provides a possible explanation for these clinical observations.

We have limited our study to investigating the expression of IL-4, IL-13 and IL-5 by inflammatory cells within the airway smooth muscle. Mast cells release in addition to cytokines a number of other vasoactive and bronchospastic mediators and proteases, which are likely to modulate smooth muscle function [31]. Future work will need to address the functional consequences that individual and combinations of mast cell cytokines and proteases have on airway smooth muscle.

In summary, mast cells localized within the airway smooth muscle in asthma express IL-4 and IL-13. Since these cytokines have the potential to exert important effects on airway smooth muscle, this observation may have major implications for our understanding of their role in the pathogenesis of asthma.

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References

- 1 Wardlaw AJ, Brightling C, Green R, Woltmann G, Pavord I. Eosinophils in asthma and other allergic diseases. *Br Med Bull* 2000; 56:985–1003.
- 2 Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med* 2002; 346:1699–705.
- 3 Brightling CE, Ward R, Goh KL, Wardlaw AJ, Pavord ID. Eosinophilic bronchitis is an important cause of chronic cough. *Am J Respir Crit Care Med* 1999; 160:406–10.
- 4 Gibson PG, Dolovich J, Denburg J, Ramsdale EH, Hargreave FE. Chronic cough: eosinophilic bronchitis without asthma. *Lancet* 1989; 1:1346–8.
- 5 Brightling CE, Ward R, Woltmann G et al. Induced sputum inflammatory mediator concentrations in eosinophilic bronchitis and asthma. *Am J Respir Crit Care Med* 2000; 162:878–82.
- 6 Gibson PG, Zlatich K, Scott J, Sewell W, Woolley K, Saltos N. Chronic cough resembles asthma with IL-5 and granulocyte-macrophage colony-stimulating factor gene expression in bronchoalveolar cells. *J Allergy Clin Immunol* 1998; 101:320–6.
- 7 Brightling CE, Symon FA, Birring SS, Bradding P, Pavord ID, Wardlaw AJ. TH2 cytokine expression in bronchoalveolar lavage fluid T lymphocytes and bronchial submucosa is a feature of asthma and eosinophilic bronchitis. *J Allergy Clin Immunol* 2002; 110:899–905.

- 8 Romagnani S. Cytokines and chemoattractants in allergic inflammation. *Mol Immunol* 2002; 38:881–5.
- 9 Bradding P, Roberts JA, Britten KM et al. Interleukin-4, -5, and -6 and tumor necrosis factor- α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 1994; 10:471–80.
- 10 Laporte JC, Moore PE, Baraldo S et al. Direct effects of interleukin-13 on signaling pathways for physiological responses in cultured human airway smooth muscle cells. *Am J Respir Crit Care Med* 2001; 164:141–8.
- 11 Rizzo CA, Yang R, Greenfeder S, Egan RW, Pauwels RA, Hey JA. The IL-5 receptor on human bronchus selectively primes for hyperresponsiveness. *J Allergy Clin Immunol* 2002; 109:404–9.
- 12 Juniper EF, Cockcroft DW, Hargreave FE. Histamine and methacholine inhalation tests: a laboratory tidal breathing protocol, 2nd Edn. Lund, Sweden: Astra Draco AB, 1994.
- 13 Pavord ID, Pizzichini MM, Pizzichini E, Hargreave FE. The use of induced sputum to investigate airway inflammation. *Thorax* 1997; 52:498–501.
- 14 British Thoracic Society. British Thoracic Society guidelines on diagnostic flexible bronchoscopy. *Thorax* 2001; 56 (Suppl. 1):i1–21.
- 15 Britten KM, Howarth PH, Roche WR. Immunohistochemistry on resin sections: a comparison of resin embedding techniques for small mucosal biopsies. *Biotech Histochem* 1993; 68:271–80.
- 16 Wilson SJ, Shute JK, Holgate ST, Howarth PH, Bradding P. Localization of interleukin (IL) -4 but not IL-5 to human mast cell secretory granules by immunoelectron microscopy. *Clin Exp Allergy* 2000; 30:493–500.
- 17 Bradding P, Okayama Y, Howarth PH, Church MK, Holgate ST. Heterogeneity of human mast cells based on cytokine content. *J Immunol* 1995; 155:297–307.
- 18 Anderson DF, Zhang S, Bradding P, McGill JJ, Holgate ST, Roche WR. The relative contribution of mast cell subsets to conjunctival TH2-like cytokines. *Invest Ophthalmol Vis Sci* 2001; 42:995–1001.
- 19 Larche M, Robinson DS, Kay AB. The role of T lymphocytes in the pathogenesis of asthma. *J Allergy Clin Immunol* 2003; 111:450–63.
- 20 Grunstein MM, Hakonarson H, Leiter J et al. IL-13 dependent autocrine signalling mediates altered responsiveness of IgE-sensitized airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2002; 282:520–8.
- 21 Espinosa K, Bosse Y, Stankova J, Rola-Pleszczynski M. CysLT1 receptor upregulation by TGF- β and IL-13 is associated with bronchial smooth muscle cell proliferation in response to LTD4. *J Allergy Clin Immunol* 2003; 111:1032–40.
- 22 Hawker KM, Johnson PR, Hughes JM, Black JL. Interleukin-4 inhibits mitogen-induced proliferation of human airway smooth muscle cells in culture. *Am J Physiol* 1998; 275:469–77.
- 23 Moore PE, Church TL, Chism DD, Panettieri RA Jr., Shore SA. IL-13 and IL-4 cause eotaxin release in human airway smooth muscle cells: a role for ERK. *Am J Physiol Lung Cell Mol Physiol* 2002; 282:L847–53.
- 24 Faffe DS, Whitehead T, Moore PE et al. IL-13 and IL-4 promote TARC release in human airway smooth muscle cells: role of IL-4 receptor genotype. *Am J Physiol Lung Cell Mol Physiol* 2003; 285:L907–14.
- 25 Hirst SJ, Hallsworth MP, Peng Q, Lee TH. Selective induction of eotaxin release by interleukin-13 or interleukin-4 in human airway smooth muscle cells is synergistic with interleukin-1 β and is mediated by the interleukin-4 receptor α -chain. *Am J Respir Crit Care Med* 2002; 165:1161–71.
- 26 de Paulis A, Annunziato F, Di Gioia L et al. Expression of the chemokine receptor CCR3 on human mast cells. *Int Arch Allergy Immunol* 2001; 124:146–50.
- 27 Romagnani P, de Paulis A, Beltrame C et al. Tryptase–chymase double-positive human mast cells express the eotaxin receptor CCR3 and are attracted by CCR3-binding chemokines. *Am J Pathol* 1999; 155:1195–204.
- 28 Borish LC, Nelson HS, Lanz MJ et al. Interleukin-4 receptor in moderate atopic asthma. A phase I/II randomized, placebo-controlled trial. *Am J Respir Crit Care Med* 1999; 160:1816–23.
- 29 Borish LC, Nelson HS, Corren J et al. Efficacy of soluble IL-4 receptor for the treatment of adults with asthma. *J Allergy Clin Immunol* 2001; 107:963–70.
- 30 Leckie MJ, ten Brinke A, Khan J et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyperresponsiveness, and the late asthmatic response. *Lancet* 2000; 356:2144–8.
- 31 Page S, Ammit AJ, Black JL, Armour CL. Human mast cell and airway smooth muscle cell interactions: implications for asthma. *Am J Physiol Lung Cell Mol Physiol* 2001; 281: L1313–23.