Published in final edited form as:
J Allergy Clin Immunol. 2008 March ; 121(3): 685–691. doi:10.1016/j.jaci.2008.01.005.

Increased sputum and bronchial biopsy IL-13 expression in severe asthma

Shironjit K. Saha, MRCP[a], **Mike A. Berry, MD, MRCP**[a], **Deborah Parker, BSc**[a], **Salman Siddiqui, MRCP**[a], **Angela Morgan, MD, MRCP**[a], **Richard May, PhD**[b], **Phillip Monk, PhD**[c], **Peter Bradding, DM, FRCP**[a], **Andrew J. Wardlaw, PhD, FRCP**[a], **Ian D. Pavord, DM, FRCP**[a], and **Christopher E. Brightling, PhD, MRCP**[a]

[a]Institute for Lung Health, Clinical Sciences Wing, University Hospitals of Leicester

[b]Cambridge Antibody Technology, Cambridge

[c]Synairgen Research Ltd, Southampton

Abstract

Background—The importance of IL-13 in the asthma paradigm is supported by increased expression in human subjects, particularly in patients with mild-to-moderate asthma. However, the role of IL-13 in severe asthma needs to be further defined.

Objective—We sought to assess IL-13 expression in sputum and bronchial biopsy specimens from subjects with mild-to-severe asthma.

Methods—Sputum IL-13 concentrations were measured in 32 control subjects, 34 subjects with mild asthma, 21 subjects with moderate asthma, and 26 subjects with severe asthma. Enumeration of mast cells, eosinophils, and IL-13$^+$ cells in the bronchial submucosa and airway smooth muscle (ASM) bundle was performed in 7 control subjects, 14 subjects with mild asthma, 7 subjects with moderate asthma, and 7 subjects with severe asthma.

Results—The proportion of subjects with measurable IL-13 in the sputum was increased in the mild asthma group (15/34) and severe asthma group (10/26) compared with that seen in the control group (4/32; $P = .004$). IL-13$^+$ cells were increased within the submucosa in all asthma severity groups compared with control subjects ($P = .006$). The number of IL-13$^+$ cells were increased within the ASM bundle in the severe asthma group compared with that seen in the other groups ($P < .05$). Asthma control questionnaire scores positively correlated with sputum IL-13 concentrations ($R_s = 0.35$, $P = .04$) and mast cells in the ASM bundle ($R_s = 0.7$, $P = .007$). IL-13$^+$ cells within the submucosa and ASM correlated with sputum eosinophilia ($R_s = 0.4$, $P \leq .05$).

Reprint requests: Christopher E. Brightling, PhD, MRCP, Institute for Lung Health, Clinical Sciences Wing, University Hospitals of Leicester, Groby Rd, Leicester, LE3 9QP, United Kingdom. ceb17@le.ac.uk.

Disclosure of potential conflict of interest: C. E. Brightling has consulting arrangements with AstraZeneca, GlaxoSmithKline, Cambridge Antibody Technology, and Roche; has received research support from AstraZeneca, Cambridge Antibody Technology, and GlaxoSmithKline; and is on the speakers' bureau for GlaxoSmithKline, AstraZeneca, and Pfizer. R. May is employed by and has equity in Cambridge Antibody Technology. P. Monk is employed by Synairgen Research Ltd. I. D. Pavord has received research support from GlaxoSmithKline and AstraZeneca. The rest of the authors have declared that they have no conflict of interest.

Clinical implications: IL-13 overexpression in severe asthma suggests that this might be an important target for novel therapies.

Europe PMC Funders Author Manuscripts

Conclusions—IL-13 overexpression in sputum and bronchial biopsy specimens is a feature of severe asthma.

Keywords

Severe asthma; IL-13; sputum; bronchus; airway smooth muscle; eosinophilia

Europe PMC Funders Author Manuscripts

Asthma is characterized by the presence of variable airflow obstruction, airway hyperresponsiveness (AHR), and an airway inflammatory response often characterized by T_H2-mediated eosinophilic airway inflammation[1] with mast cell infiltration of the airway smooth muscle (ASM) bundle.[2] Comparisons between asthma and nonasthmatic eosinophilic bronchitis, a common cause of chronic cough,[3] have been informative about the key immunopathologic features of asthma. Importantly, overexpression of the T_H2 cytokine IL-13 in sputum,[4,5] bronchial submucosa,[4] peripheral blood,[6] and colocalization to mast cells in the ASM bundle[7] are features of asthma that are not shared by eosinophilic bronchitis and have therefore been implicated in the pathogenesis of AHR.

A role for IL-13 in the asthma paradigm is further supported by other human studies that have reported increased IL-13 mRNA expression in bronchial biopsy specimens from subjects with moderate asthma[8,9] and from sputum cells from corticosteroidnaive and inhaled corticosteroid treated–asthmatic subjects.[10] In addition, after allergen challenge in subjects with mild asthma, bronchoalveolar lavage IL-13 concentrations were upregulated.[11] This association between IL-13 and asthma in human subjects is supported by animal models.[12] T lymphocyte–deficient mice have shown that exogenous addition of IL-13 promotes AHR and airway inflammation, whereas neutralization of IL-13 in murine models can resolve these features.[13]

To date, human studies have focused their investigation on subjects with mild-to-moderate asthma.[4-9,11] Therefore whether IL-13 expression is associated with severe refractory disease[14] is unclear. Refractory asthma accounts for a large proportion of the morbidity, mortality, and health care costs associated with this disease. Thus there is a pressing need to identify and test novel targets in this group of patients.

We hypothesized that in addition to mild asthma, increased IL-13 expression is a feature of severe refractory asthma. To test our hypothesis, we measured the sputum IL-13 concentration and the number of $IL\text{-}13^+$ cells in the bronchial submucosa and ASM bundle in a cross-sectional study that included subjects with mild, moderate, and severe refractory asthma and healthy control subjects. To further define the possible role of IL-13 in asthma, we investigated the relationship between IL-13 expression and disease severity, asthma control, AHR, spirometric results, and eosinophilic inflammation.

METHODS

Subjects

Subjects were recruited from local primary health care providers, respiratory clinics, hospital staff, and through local advertising. Asthma was defined and severity categorized by using international (Global Initiative for Asthma [GINA]) guidelines[15] and American Thoracic

Society criteria for refractory asthma.[14] Healthy subjects had no history of respiratory symptoms and normal spirometric results. All subjects provided written informed consent, with study approval from the Leicestershire ethics committee.

Subjects were recruited as 2 independent cross-sectional cohorts, except for 2 subjects who were included in both cohorts, to assess IL-13 expression in sputum (cohort 1) and bronchial biopsy specimens (cohort 2). Forty-four of 109 subjects with asthma and 18 of 39 healthy control subjects had participated in earlier studies.[4,16]

Clinical characterization

Subjects underwent spirometry; allergen skin prick tests for *Dermatophagoides pteronyssinus*, dog, cat, and grass pollen; a methacholine inhalation test using the tidal breathing method[17]; and sputum induction using incremental concentrations of nebulized hypertonic saline (ie, 3%, 4%, and 5%, each for 5 minutes).[18] Subjects with a sputum eosinophil count of greater than 3% were defined as having eosinophilic asthma. In those subjects with moderate-to-severe disease, symptom control was assessed by using the Juniper Asthma Control Questionnaire (ACQ).[19]

Cohort 1: Sputum IL-13 measurement

Subjects with asthma were categorized as having mild (GINA class 1, n = 34), moderate (GINA class 2-4, n = 21), or severe (GINA class 5, n = 26) disease. All the subjects in the severe group also fulfilled the criteria for severe refractory asthma.[14] Eleven of 26 of these subjects with severe asthma were treated with intramuscular triamcinolone based on clinical grounds because of symptoms deemed unresponsive to oral corticosteroid therapy.

Sputum IL-13 was measured by using a validated ELISA (Caltag-Medsystems, Buckinghamshire, United Kingdom), as described previously.[4] The lower limit of detection was 10 pg/g sputum.

Cohort 2: IL-13 measurement in endobronchial biopsy specimens

Subjects with assessable ASM (>0.1 mm^2) in bronchial biopsy specimens were recruited.[2] Asthma was categorized as mild (GINA class 1, n = 14), moderate (GINA class 2-3, n = 7), or severe (GINA class 4-5, n = 7). All of the subjects in the severe asthma category had severe refractory asthma.[14] To examine IL-13 expression in noneosinophilic asthma, we included 7 asthmatic subjects in GINA class 1 without a sputum eosinophilia (less than 1.9% on 2 separate occasions). In this cohort we chose to specifically compare corticosteroid-naive subjects with those with eosinophilic and noneosinophilic asthma to exclude the possible confounder of treatment and applied a rigorous definition for noneosinophilic asthma.[16]

After characterization, subjects underwent bronchoscopy conducted according to the British Thoracic Society guidelines.[20] Bronchial mucosal biopsy specimens were taken from the right middle lobe and lower lobe carinae, fixed in acetone, and embedded in glycomethacrylate, as described previously.[21]

Two-micrometer sections were cut and stained with mAbs against IL-13 (R&D systems, Oxfordshire, United Kingdom), tryptase for mast cells (DAKO UK, Cambridgeshire, United Kingdom), major basic protein for eosinophils (Monosan, Uden, the Netherlands), or appropriate isotype controls (DAKO). The number of positive nucleated cells was enumerated per square millimeter of bronchial submucosa or ASM bundle by a blinded observer. Sequential sections were stained for IL-13 and tryptase or major basic protein to assess colocalization, as described previously.[4,7]

Statistical analysis

Statistical analysis was performed with PRISM version 4 and MINI-TAB13.31 (Minitab, Coventry, United Kingdom). Parametric data were expressed as the mean (SEM), data that had a normal log distribution were log transformed and described as the geometric mean (log SE), and nonparametric data were described as the median (interquartile range). One-way ANOVA and *t* tests (Kruskal-Wallis and Mann-Whitney tests for nonparametric data) were used for across- and between-group comparisons, respectively. χ^2 Tests were used to compare categorical data. Correlations were assessed by using Spearman rank correlation coefficients.

RESULTS

Clinical and sputum characteristics for subjects in cohort 1 are shown in Table I. The groups with asthma were well matched for AHR and sputum eosinophilic inflammation. The sputum IL-13 concentration for each subject is shown in Fig 1. The proportion of subjects with measurable IL-13 in their sputum supernatant was increased in those with severe asthma (10/26) and mild asthma (15/34) compared with the proportion of healthy control subjects (4/32, $P < .05$). In addition, the proportion of subjects with measurable IL-13 in the mild asthma group was increased compared with that in the moderate asthma group (3/21, $P = .022$). Among the 11 subjects with severe asthma requiring treatment with intramuscular triamcinolone, 6 had measurable IL-13 in their sputum ($P = .01$ compared with healthy control subjects). The sputum IL-13 concentration was increased in those with mild asthma compared with those with moderate disease ($P = .04$) and control subjects ($P < .01$). The sputum IL-13 concentration was increased in the severe asthma group when compared with that in the control group ($P = .027$) but was not significantly increased compared with that in the moderate disease group ($P = .059$).

There was no significant correlation between sputum IL-13 concentration and any of the sputum differential cell counts, FEV_1, or AHR in the asthmatic subjects. Sputum IL-13 levels exhibited a significant positive correlation with ACQ scores ($R_s = 0.35$, $P = .04$) for subjects with moderate and severe asthma. In these 2 groups subjects with detectable IL-13 had higher ACQ scores (3.2 [1.4]) compared with subjects with immeasurable IL-13 (2.1 [1.7], $P = .05$).

Clinical and sputum characteristics for subjects in cohort 2 are shown in Table II, and the number of mast cells, eosinophils, and IL-13^+ cells in the bronchial submucosa and ASM bundle are shown in Table III. Representative photomicrographs of IL-13^+ cells in the submucosa and ASM bundle are as shown in Fig 2.

The number of mast cells within the ASM bundle in asthmatic subjects was increased compared with the number in the control subjects, irrespective of disease severity (P = .009; Fig 3, *A*). The number of mast cells in the ASM bundle was increased in the subjects with mild eosinophilic asthma (11.3 [3.4]) compared with that in the subjects with mild noneosinophilic asthma (7.5 [5.8], P = .018).

The number of IL-13$^+$ cells in the bronchial submucosa was increased in all asthma severity groups in comparison with that in the healthy control subjects (P = .006; Fig 3, *B*, and Table III). The mean (SEM) proportion of IL-13$^+$ cells in the submucosa colocalized to mast cells was 22% (4%), and that to eosinophils was 66% (6%). There were no differences across disease severity. In the ASM bundle the number of IL-13$^+$ cells was increased in subjects with mild and severe asthma compared with that seen in the healthy control group (P <.01, Table III). The number of IL-13$^+$ cells in the ASM bundle was increased in the subjects with severe asthma in comparison with that seen in the subjects with mild asthma (P = .027) and moderate asthma (P = .007; Fig 3, *C*). The number of IL-13$^+$ cells in the ASM bundle was increased in the subjects with mild eosinophilic asthma (2.2 [1.57]) compared with that in the subjects with mild noneosinophilic asthma (0 [0], P = .002; Fig 3, *C*). The mean (SEM) proportion of IL-13$^+$ cells in the ASM bundle that were colocalized to mast cells was 99% (0.8%), and that to eosinophils was 0%. This was not different across disease severity. There was no significant correlation between the number of IL-13$^+$ cells in either the ASM bundle or submucosa and FEV_1 percent predicted or AHR.

The number of mast cells within the ASM bundle was related to asthma control (R_s = 0.7, P = .007). The ACQ scores in those subjects with IL-13$^+$ cells in the ASM bundle were not significantly higher than those in subjects without IL-13$^+$ cells (2.4 [2.1] vs 1.9 [2.0], P = .5). There was no significant correlation between ACQ scores and IL-13 expression within the submucosa and ASM.

The number of IL-13$^+$ cells within the submucosa positively correlated with the sputum eosinophil count across all asthma disease groups (R_s = 0.42, P = .042). The number of IL-13$^+$ cells within the ASM bundle positively correlated with sputum eosinophil count (R_s = 0.40, P = .05) and the number of eosinophils in the submucosa (R_s = 0.39, P = .038).

DISCUSSION

For the first time, we have shown that sputum IL-13 concentration and the number of IL-13$^+$ cells in the bronchial submucosa and ASM bundle were increased in severe asthma. We have confirmed our earlier observation that mast cell localization to the ASM bundle is a feature of mild asthma[2] and demonstrated for the first time that this is also characteristic of moderate and severe refractory disease. Interestingly, in contrast to severe asthma, increased sputum IL-13 concentrations and IL-13$^+$ cells in the ASM bundle were not observed in subjects with moderate disease. IL-13 expression within the submucosa and ASM bundle was positively correlated to the intensity of eosinophilic airway inflammation, and sputum IL-13 concentration was related to asthma control, as determined by using the ACQ. Sputum and bronchial biopsy specimen expression of IL-13 was not related to FEV_1 or AHR.

There is compelling evidence implicating IL-13 as a central mediator in the pathogenesis of asthma from studies using animal models and in human disease.[22] A number of reports describe an association between polymorphisms in the IL-13 gene with aspects of the asthma phenotype.[23,24] In subjects with mild-to-moderate asthma, but not in subjects with nonasthmatic eosinophilic bronchitis, IL-13 levels were increased in bronchoalveolar lavage fluid, bronchial biopsy specimens, and sputum.[4,6-8] Similarly, IL-13 mRNA expression was increased in sputum cells from corticosteroid-naive and inhaled corticosteroid–treated asthmatic subjects.[10] We now provide evidence to support a role for IL-13 in severe refractory disease. Sputum IL-13 concentrations and the numbers of IL-13$^+$ cells in bronchial biopsy specimens were increased in severe disease. However, our data do suggest that the relationship between IL-13 expression in sputum and bronchial biopsy specimens, disordered airway physiology, and asthma control is complex. We were unable to demonstrate a correlation between IL-13 expression and AHR or FEV_1. In contrast to severe disease, IL-13 expression was not increased in moderate disease. The relative lack of IL-13 expression in this group of subjects with moderate asthma is likely to reflect a favorable response to corticosteroid therapy. However, these subjects had persistent AHR, suggesting that AHR and IL-13 expression can be disassociated. Interestingly, sputum IL-13 concentration was related to asthma control. Severe refractory asthma is characterized by poor control, recurrent exacerbations, and the development of persistent airflow obstruction. The rates of death and complications are high among patients with severe refractory asthma, and these patients account for a disproportionate amount of the health care cost attributed to asthma.[14] There is therefore a significant unmet need in this group of asthmatic subjects. Whether therapies targeted at IL-13 are effective in subjects with severe refractory asthma needs to be investigated.

Consistent with the view that IL-13 is associated with eosinophilic airway inflammation, we found that IL-13 expression in the bronchial biopsy specimens was positively correlated with eosinophilic inflammation in sputum and biopsy specimens. We were unable to demonstrate a relationship between sputum IL-13 concentration and sputum eosinophil counts. However, this was perhaps not surprising because less than 50% of the subjects had measurable IL-13 in their sputum. In our group of subjects with mild asthma, we included a group of well-characterized subjects with noneosinophilic asthma. These subjects had failed to demonstrate eosinophilic inflammation in their sputum on repeated occasions. The inclusion of this group gave us an opportunity to examine IL-13 expression in tissue in a group of subjects with noneosinophilic asthma without the potential confounder of corticosteroid therapy. We found the numbers of IL-13$^+$ cells in the ASM bundle were markedly reduced in those subjects with noneosinophilic asthma. The identification of differential expression of IL-13 in eosinophilic and noneosinophilic asthma is important because it suggests there are fundamental differences in the underlying pathogenesis of these disease phenotypes, and this might be important in patient selection for the use of novel therapies in asthma.

Mast cell microlocalization to the ASM bundle is a feature of asthma across severities.[2,25-29] In subjects with mild asthma, mast cells in the ASM bundle express IL-4 and IL-13.[7] In this study we found that the number of IL-13$^+$ cells in the ASM bundle was increased in subjects with mild and severe asthma, and in keeping with our earlier report,[7] the vast majority of

these cells were mast cells. Many cytokines exert their effects across distances of a few microns.[1] The immediate proximity of the IL-13$^+$ cells and ASM is therefore likely to be functionally important. ASM is an important source of proinflammatory mediators, such as CCL11, and IL-13 induces the ASM synthesis and release of this and other chemokines.[30-32] *In vitro* IL-13, but not IL-4, has been shown to attenuate ASM relaxation to β-agonists[33] and augment contractility to acetylcholine,[34] suggesting that IL-13 might induce AHR by directly activating ASM. Mast cell–derived IL-13 in the ASM bundle has the potential to promote IgE-mediated mast cell activation and proliferation through an autocrine mechanism.[35] Hence the location of IL-13$^+$ cells in the ASM bundle and the consequent IL-13/ASM interactions might contribute to the pathogenesis of severe asthma.

One criticism of our study is the cross-sectional design. We have not assessed the response to corticosteroids within individuals, and therefore we do not know whether the IL-13 expression in mild disease is corticosteroid responsive, as suggested by the relative lack of IL-13 expression in subjects with moderate disease. However, an earlier report found that in patients who were clinically corticosteroid responsive, treatment with oral corticosteroid for 1 week led to a reduction in IL-13 mRNA expression in bronchial biopsy specimens, whereas in those subjects who were clinically corticosteroid nonresponsive, IL-13 mRNA expression persisted after treatment.[8] This is entirely consistent with our view that IL-13 expression is attenuated in those asthmatic subjects with moderate disease whose symptoms are adequately controlled with inhaled corticosteroids. This apparent shortcoming of our study design does not detract from our observation that severe disease was associated with IL-13 expression. In addition, although the measurement of IL-13 in sputum is limited by the sensitivity of the assay, with several subjects having undetectable sputum IL-13 levels, we are confident that this observation is robust because it was confirmed in 2 cohorts in sputum and in bronchial biopsy specimens. Importantly, sputum IL-13 concentrations were increased, even in subjects treated with intramuscular triamcinolone, excluding the possibility of poor adherence to therapy.

In conclusion, IL-13 overexpression is a feature of many patients with severe asthma with increased sputum IL-13 concentration and IL-13$^+$ cells in the bronchial submucosa and ASM bundle. IL-13 expression was related to asthma control and the intensity of eosinophilic inflammation but not to the severity of disordered airway physiology. We suggest that IL-13 might have an important role in the pathophysiology of severe asthma, and future studies targeted at the IL-13 axis are eagerly awaited.

Acknowledgments

We thank Will Monteiro for the processing and analysis of sputum samples, Natalie Neale and colleagues for their assistance with immunohistochemical staining of biopsy tissue, and Beverley Hargadon and colleagues for help with patient clinical characterization.

Supported by Asthma UK, Cambridge Antibody Technology, GlaxoSmithKline, and a DOH Clinical Scientist award.

Abbreviations used

ACQ	Juniper Asthma Control Questionnaire
AHR	Airway hyperresponsiveness
ASM	Airway smooth muscle
GINA	Global Initiative for Asthma

REFERENCES

1. Wardlaw AJ, Brightling CE, Green R, Woltmann G, Bradding P, Pavord ID. New insights into the relationship between airway inflammation and asthma. Clin Sci (Lond). 2002; 103:201–11. [PubMed: 12149112]
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. [PubMed: 12037149]
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. [PubMed: 10430705]
4. Berry MA, Parker D, Neale N, Woodman L, Morgan A, Monk P, et al. and bronchial submucosal IL-13 expression in asthma and eosinophilic bronchitis. J Allergy Clin Immunol. 2004; 114:1106–9. [PubMed: 15536417]
5. Komai-Koma M, McKay A, Thomson L, McSharry C, Chalmers GW, Liew FY, et al. Immuno-regulatory cytokines in asthma: IL-15 and IL-13 in induced sputum. Clin Exp Allergy. 2001; 31:1441–8. [PubMed: 11591195]
6. Park SW, Jangm HK, An MH, Min JW, Jang AS, Lee JH, et al. Interleukin-13 and interleukin-5 in induced sputum of eosinophilic bronchitis: comparison with asthma. Chest. 2005; 128:1921–7. [PubMed: 16236836]
7. Brightling CE, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID, Bradding P. Interleukin-4 and -13 expression is co-localized to mast cells within the airway smooth muscle in asthma. Clin Exp Allergy. 2003; 33:1711–6. [PubMed: 14656359]
8. Naseer T, Minshall EM, Leung DY, Laberge S, Ernst S, Martin RJ, et al. Expression of IL-12 and IL-13 mRNA in asthma and their modulation in response to steroid therapy. Am J Respir Crit Care Med. 1997; 155:845–51. [PubMed: 9117015]
9. Humbert M, Durham SR, Kimmitt P, Powell N, Assoufi B, Pfister R, et al. Elevated expression of messenger ribonucleic acid encoding IL-13 in the bronchial mucosa of atopic and nonatopic subjects with asthma. J Allergy Clin Immunol. 1997; 99:657–65. [PubMed: 9155833]
10. Truyen E, Coteur L, Dilissen E, Overbergh L, Dupont LJ, Ceuppens JL, et al. Evaluation of airway inflammation by quantitative Th1/Th2 cytokine mRNA measurement in sputum of asthma patients. Thorax. 2006; 61:202–8. [PubMed: 16449261]
11. Huang SK, Xiao HQ, Kleine-Tebbe J, Paciotti G, Marsh DG, Lichtenstein LM, et al. IL-13 expression at the sites of allergen challenge in patients with asthma. J Immunol. 1995; 155:2688–94. [PubMed: 7650396]
12. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. J Clin Invest. 1999; 103:779–88. [PubMed: 10079098]
13. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. Science. 1998; 282:2261–3. [PubMed: 9856950]
14. Proceedings of the ATS Workshop on Refractory Asthma. Current understanding, recommendations, and unanswered questions. Am J Respir Crit Care Med. 2000; 162:2341–51. [PubMed: 11112161]
15. [Accessed February 1, 2008] Global Initiative for Asthma Guidelines 11/06. Available at: www.ginasthma.com

16. Berry MA, Morgan A, Shaw DE, Parker D, Green RH, Brightling CE, et al. Pathological features and inhaled corticosteroid response of eosinophilic and noneosinophilic asthma. Thorax. 2007; 62:1043–9. [PubMed: 17356056]
17. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care Med. 2000; 161:309–29. [PubMed: 10619836]
18. Djukanovic R, Sterk PJ, Fahy JV, Hargreave FE. Standardised methodology of sputum induction and processing. Eur Respir J Suppl. 2002; 37:1s–2s. [PubMed: 12361359]
19. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. Eur Respir J. 1999; 14:902–7. [PubMed: 10573240]
20. British Thoracic Society. British Thoracic Society guidelines on diagnostic flexible bronchoscopy. Thorax. 2001; 56(suppl 1):i1–21. [PubMed: 11158709]
21. 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. [PubMed: 8268322]
22. Wills-Karp M, Chiaramonte M. Interleukin-13 in asthma. Curr Opin Pulm Med. 2003; 9:21–7. [PubMed: 12476080]
23. Howard TD, Whittaker PA, Zaiman AL, Koppelman GH, Xu J, Hanley MT, et al. Identification and association of polymorphisms in the interleukin-13 gene with asthma and atopy in a Dutch population. Am J Respir Cell Mol Biol. 2001; 25:377–84. [PubMed: 11588017]
24. van der Pouw Kraan TC, van Veen A, Boeije LC, van Tuyl SA, de Groot ER, Stapel SO, et al. An IL-13 promoter polymorphism associated with increased risk of allergic asthma. Genes Immun. 1999; 1:61–5. [PubMed: 11197307]
25. Koshino T, Teshima S, Fukushima N, Takaishi T, Hirai K, Miyamoto Y, et al. Identification of basophils by immunohistochemistry in the airways of post-mortem cases of fatal asthma. Clin Exp Allergy. 1993; 23:919–25. [PubMed: 10779279]
26. Carroll NG, Mutavdzic S, James AL. Distribution and degranulation of airway mast cells in normal and asthmatic subjects. Eur Respir J. 2002; 19:879–85. [PubMed: 12030728]
27. Berger P, Girodet PO, Begueret H, Ousova O, Perng DW, Marthan R, et al. Tryptase-stimulated human airway smooth muscle cells induce cytokine synthesis and mast cell chemotaxis. FASEB J. 2003; 17:2139–41. [PubMed: 14500550]
28. Amin K, Janson C, Boman G, Venge P. The extracellular deposition of mast cell products is increased in hypertrophic airways smooth muscles in allergic asthma but not in nonallergic asthma. Allergy. 2005; 60:1241–7. [PubMed: 16134989]
29. El Shazly A, Berger P, Girodet PO, Ousova O, Fayon M, Vernejoux JM, et al. Fraktalkine produced by airway smooth muscle cells contributes to mast cell recruitment in asthma. J Immunol. 2006; 176:1860–8. [PubMed: 16424217]
30. Sutcliffe A, Kaur D, Page S, Woodman L, Armour CL, Baraket M, et al. Mast cell migration to Th2 stimulated airway smooth muscle from asthmatics. Thorax. 2006; 61:657–62. [PubMed: 16601090]
31. 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-1beta and is mediated by the interleukin-4 receptor alpha-chain. Am J Respir Crit Care Med. 2002; 165:1161–71. [PubMed: 11956062]
32. Peng Q, Matsuda T, Hirst SJ. Signaling pathways regulating interleukin-13-stimulated chemokine release from airway smooth muscle. Am J Respir Crit Care Med. 2004; 169:596–603. [PubMed: 14670803]
33. Laporte JC, Moore PE, Baraldo S, Jouvin MH, Church TL, Schwartzman IN, 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. [PubMed: 11435252]
34. Grunstein MM, Hakonarson H, Leiter J, Chen M, Whelan R, Grunstein JS, 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.

35. Kaur D, Hollins F, Woodman L, Yang W, Monk P, May R, et al. Mast cells express IL-13R alpha 1: IL-13 promotes human lung mast cell proliferation and Fc epsilon RI expression. Allergy. 2006; 61:1047–53. [PubMed: 16918506]

Europe PMC Funders Author Manuscripts

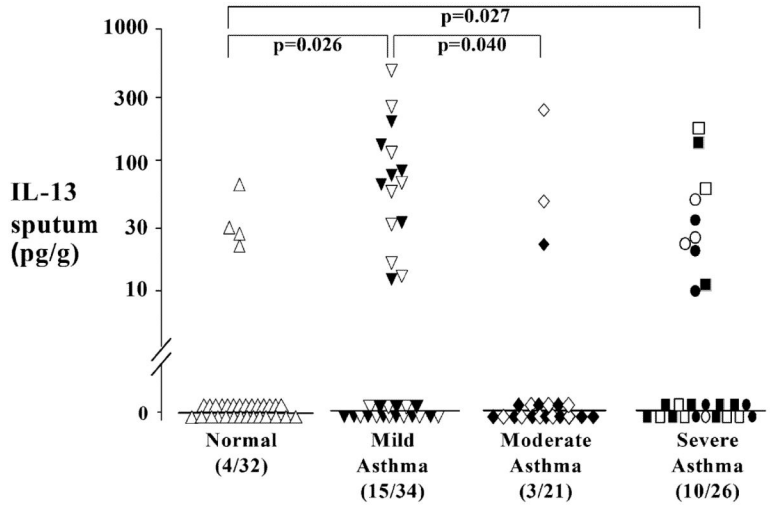

FIG 1.

Sputum IL-13 concentration. Δ, Sputum IL-13 concentration in the control group; ▼, mild asthma (GINA class 1); ◆, moderate asthma (GINA class 2-4); ■, severe asthma (GINA class 5) oral corticosteroid; ●, severe asthma (GINA class 5) intramuscular triamcinolone. *Solid symbols* indicate sputum eosinophilia greater than 3%. *Horizontal bars* represent median value. *Values in parentheses* represent subjects with measurable IL-13/total subjects in group. *P* = .012, Kruskal-Wallis test. *P* values on the figure are from the Mann-Whitney test.

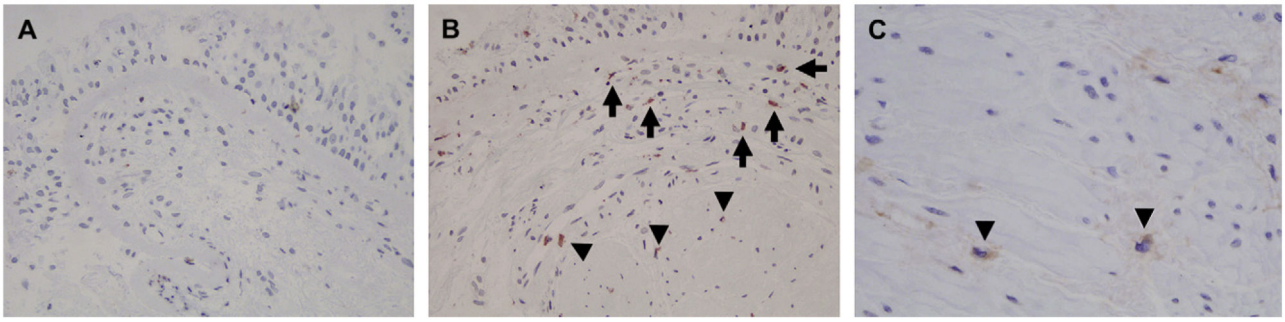

FIG 2.

Examples of IL-13$^+$ cells in the submucosa and ASM bundle in subjects with asthma. Representative photomicrographs of bronchial biopsy sections from a subject with severe asthmatic illustrating isotype control (**A**; original magnification ×200), IL-13$^+$ cells present in the bronchial submucosa and ASM bundle (**B**; original magnification ×200), and IL-13$^+$ cells within the ASM bundle (**C**; original magnification ×400) are shown. IL-13$^+$ cells are highlighted in the submucosa by *arrows* and in the ASM bundle by *arrowheads*.

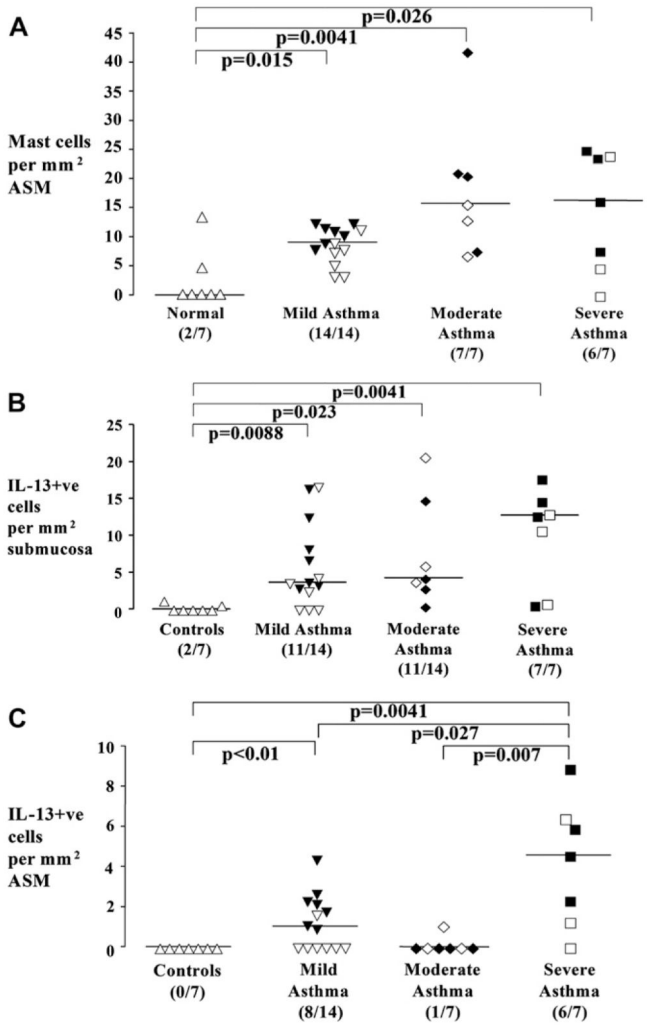

FIG 3.

The number of IL-13$^+$ cells in the submucosa and ASM bundle in subjects with asthma. The number of mast cells in the ASM bundle (**A**), IL-13$^+$ cells in the bronchial submucosa (**B**), and IL-13$^+$ cells in the ASM bundle (**C**) are shown. Δ, Mild asthma (GINA class 1); ▼, moderate asthma (GINA class 2-3); ◆, severe asthma (GINA class 4-5); ■. *Solid symbols* represent sputum eosinophilia greater than 3%. *Horizontal bars* represent median value. *Values in parentheses* represent subjects with mast cells or IL-13$^+$ cells present in ASM or submucosa/total subjects in group.

TABLE I

Clinical and sputum characteristics of cohort 1

	Healthy control subjects	Subjects with mild asthma	Subjects with moderate asthma	Subjects with severe asthma
No.	32	34	21	26
ICS use (%)	0	0	100	100
ICS dose (μg/d)*	0	0	1575 (224.1)	1704 (95.7)
LABA use (%)	0	0	52	92
Systemic CS use (%)	0	0	0	100
Oral/intramuscular CS dose (mg/d)*	0	0	0	10.3 (2.2)/54.6 (6.1)
Age (y)*	47.6 (3.0)	48.5 (0.1)	49.5 (3.4)	48.3 (3.4)
Male sex	15	16	9	11
Never smokers	25	31	16	23
Pack-years (all subjects)*	8.2 (2.9)	1.7 (0.8)	5.1 (2.3)	3.5 (2.2)
Atopy (%)	24	44	87	74
PC_{20} FEV_1 (mg/mL)†	>16	1.3 (0.12)	0.36 (0.2)	0.25 (0.4)
FEV_1 % predicted*	96.4 (2.9)	82.4 (4.3)‡	66.6 (6.7)§	59.6 (4.3)‖
Bronchodilator reversibility (%)*	ND	5.8 (2.2)	6.5 (3.5)	8.6 (2.0)
FEV_1/FVC (%)*	78.7 (1.7)	72.0 (1.9)	68.6 (2.8)	66.9 (2.7)
Sputum cell counts				
Eosinophil (%)†¶	0.5 (0.1)	2.3 (0.1)¶	2.9 (0.2)¶	3.6 (0.1)¶
Neutrophil (%)*	46.6 (4.6)	61.6 (4.3)	59.7 (37.0)	64.5 (4.3)
Macrophage (%)*¶	48.3 (4.6)	28.0 (3.7)¶	26.5 (18.7)¶	17.7 (2.5)¶
Lymphocyte (%)*	1.8 (0.4)	1.1 (0.2)	0.4 (1.5)	0.4 (0.1)
Epithelial cells (%)*	3.5 (1.0)	3.1 (0.7)	2.3 (5.5)	5.6 (1.4)

ICS, Inhaled corticosteroid; *LABA*, long-acting β-agonist; *CS*, corticosteroid; *ND*, not done; *FVC*, forced vital capacity.

*Mean (SE).

†Geometric mean (log SE).

‡$P < .01$, mild versus severe groups.

§$P < .001$, healthy versus moderate groups.

‖$P < .001$, healthy versus severe groups (Tukey multiple comparison test).

¶$P < .0001$ (ANOVA), healthy versus mild/moderate/severe groups.

TABLE II

Clinical and sputum characteristics of cohort 2

	Healthy control subjects	Subjects with mild asthma	Subjects with moderate asthma	Subjects with severe asthma
No.	7	14	7	7
ICS use (%)	0	0	100	100
ICS dose (μg/d)*	0	0	942.9 (36.9)	1520.0 (224.5)‡
Oral CS use (%)	0	0	0	71
Oral CS dose (mg/d)*	0	0	0	15.0 (5.4)
LABA use (%)	0	0	100	100
Age (y)*	37.6 (7.3)	52.1 (3.8)	43.4 (4.7)	46.4 (2.7)
Male sex	7	8	3	3
Never smokers	7	14	7	6
Pack-years (all subjects)*	0	0	0.83 (0.83)	3.4 (2.1)
Atopy (%)	14	50	86	71
PC_{20} FEV_1 (mg/mL)†	>16	0.79 (0.21)	0.66 (0.5)	0.47 (0.4)
FEV_1 % predicted*	106.6 (6.6)	95.3 (3.8)§	81.3 (8.4)§	74.3 (11.3)§
Bronchodilator reversibility (%)*	ND	4.4 (1.7)	6.3 (4.0)	15 (6.8)
FEV_1/FVC (%)*	81.7 (2.7)	71.2 (8.7)	76.3 (1.8)	70.9 (4.9)
Sputum cell counts				
Eosinophil (%)t	0.39 (0.15)	1.70 (0.2)	5.7 (0.29)‖	7.7 (0.21)‖
Neutrophil (%)*	38.7 (7.2)	60.6 (8.4)	41.4 (14.7)	34.5 (9.7)
Macrophage (%)*	53.6 (5.7)	28.2 (8.2)	35.9 (8.6)	46.2 (12.7)
Lymphocyte (%)*	4.2 (2.0)	0.59 (0.13)	1.6 (0.42)	1.1 (0.30)
Epithelial cells (%)*	6.0 (3.2)	3.2 (1.2)	7.4 (3.5)	2.84 (1.8)

ICS, Inhaled corticosteroid; *CS*, corticosteroid; *LABA*, long-acting (β-agonist; *ND*, not done; *FVC*, forced vital capacity.

*Mean (SE).

†Geometric mean (log SE).

‡$P < .05$, moderate versus severe groups (Mann-Whitney).

§$P < .001$, healthy versus moderate/severe groups; $P < .01$ mild versus severe groups (Tukey multiple comparison test).

‖$P < .01$ (ANOVA).

Europe PMC Funders Author Manuscripts

TABLE III

Median (interquartile range) inflammatory cell infiltration of submucosa and ASM

	Healthy control subjects	Subjects with mild asthma	Subjects with moderate asthma	Subjects with severe asthma
Submucosa cells/mm^2				
Tryptase$^+$	16.4 (18.0)	21.8 (22.1)	24 (28.4)	21.5 (29.1)
MBP1$^+$*	2.5 (7.4)	11.9 (25.9)*	8.1 (22.1)	21.8 (27.4)*
IL-13$^+$†	0(0)	3.7 (9.1)†	4.3 (12.0)†	12.7 (13.9)†
ASM cells/mm^2				
Tryptase$^+$‡	0 (0)	9.1 (5.1)‡	15.7 (13.5)‡	16.2 (19.3)‡
MBP1$^+$	0	0	0	0 (0.42)
IL-13$^+$§	0 (0)	1.0 (2.2)§	0 (0)	4.6 (5.2)§

MBP, Major basic protein.

*$P < .05$, healthy versus mild and severe groups (Mann-Whitney).

†$P < .01$, healthy versus other groups (Kruskal-Wallis).

‡$P < .05$, healthy versus mild and severe groups; $P < .01$, healthy versus moderate groups (Mann-Whitney).

§$P < .05$, mild versus severe groups; $P < .01$, moderate versus severe groups.