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Induced sputum and bronchial mucosal expression of interleukin-13 is not increased in chronic obstructive pulmonary disease

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Abstract

Background—The Th2 cytokine interleukin-13 (IL-13) has been implicated in the pathogenesis of chronic obstructive pulmonary disease (COPD). We sought to examine IL-13 expression in COPD subjects in induced sputum and bronchus specimens. We hypothesized that inflammatory cells expressing IL-13 localize to the airway smooth muscle bundle and bronchial glands.

Methods—Interleukin-13 was measured in sputum samples from subjects with COPD ($n = 34$) across a range of severity (Global initiative for chronic Obstructive Lung Disease 2–4) and controls ($n = 14$) using ELISA. IL-13+ cells and inflammatory cells were enumerated within surgically resected proximal airway using immunohistochemical techniques from subjects with COPD ($n = 10$), smoking ($n = 10$) and nonsmoking controls ($n = 8$).

Results—Sputum IL-13 was measurable in only 6/34 subjects with COPD and was not found in the smoking or nonsmoking control subjects. In subjects with COPD and controls there was a paucity of IL-13+ cells. The distribution of inflammatory cells within different airway compartments was similar in COPD and controls except for an increase in CD3⁺ lymphocytes within bronchial glands in COPD ($P = 0.04$).

Conclusions—Our findings do not support a role for IL-13 in COPD. However, the tissue localization of inflammatory cells to airway compartments, particularly the increase of T cells in glands in COPD may be important in disease.

Keywords

airway smooth muscle; bronchus; chronic obstructive pulmonary disease; interleukin-13; sputum

Chronic obstructive pulmonary disease (COPD) is a progressive condition characterized by fixed airflow obstruction with airway inflammation, remodelling of the large and small airways, and peripheral alveolar destruction (1). Some of the inflammatory changes within the airways are mediated through cytokines and chemokines secreted locally from recruited inflammatory cells.

Interleukin-13 (IL-13), a Th2 cytokine, is found within T lymphocytes, mast cells, eosinophils, basophils and macrophages and has been implicated in recruitment of inflammatory cells from the blood to lung tissue, regulation of matrix metalloproteinases, and IgE production (2). Transgenic mouse models suggest a central role for IL-13 in phenotypes mimicking aspects of COPD (3, 4). In contrast, data from human studies have been conflicting. IL-13 expression within macroscopic emphysematous lung tissue was low in severe emphysema (5), whereas IL-13 expression was increased within the proximal airway submucosa in chronic bronchitis (6).

Localization of inflammatory cells to airway structural components is likely to be a critical disease feature as it facilitates cellular cross-talk (7). For example localization of mast cells within the airway smooth muscle (ASM)-bundle has been implicated in development of the disordered airway physiology observed in asthma (7, 8). The location of inflammatory cells in different proximal airway structures in COPD has not been fully examined and whether these cells express IL-13 remains uncertain.

We hypothesized that in COPD inflammatory cells express elevated IL-13 and are increased in the submucosal glands and ASM-bundle. To test our hypothesis, we examined the concentration of IL-13 in induced sputum samples and the number of inflammatory cells and IL-13 expression in the proximal airway structural compartments from subjects with COPD, and smoking and nonsmoking controls.

Materials and methods

Subjects

Subjects were recruited from local primary health care, respiratory clinics, hospital staff and by local advertising. COPD was diagnosed and severity categorized by using Global initiative for chronic Obstructive Lung Disease (GOLD) criteria (9). Subjects were recruited as two independent cross-sectional cohorts, to assess IL-13 expression in sputum (cohort 1) and proximal airways (cohort 2). All subjects gave written informed consent with study approval from the Leicestershire ethics committee.

Smoking history and spirometry were recorded in all subjects. In cohort 1 subjects also underwent sputum induction (10), bronchodilator reversibility 15 min after administration of salbutamol 400 µg via a volumatic, skin prick tests for common aeroallergens and total peripheral blood IgE and the presence or absence of chronic bronchitis was recorded. Healthy controls for cohort 1 ($n = 14$) were defined by an absence of respiratory symptoms with normal spirometry. In cohort 2, large airway tissue from surgical specimens was obtained from 10 COPD subjects (GOLD stages 1–2), and 18 non-COPD controls. The

controls consisted of 10 subjects with, and eight subjects without, >10 pack year smoking history with normal spirometry.

IL-13 measurement in sputum

Sputum IL-13 was measured by a validated ELISA (Bender-Med Caltag Systems, Buckinghamshire, UK) as described previously (10). Sputum from asthmatics were also measured at the same time as samples from this study and acted as positive controls (11). The lower limit of detection was 10 pg/g sputum.

Immunohistochemical assessment of proximal airway

Proximal airway samples were collected from surgical specimens, fixed in acetone, and embedded in glycomethacrylate as described previously (8, 10). Two micrometre sections were cut and stained using monoclonal antibodies against IL-13 (R&D, Abingdon, UK), tryptase (Dako UK, Ely, UK), major basic protein (Monosan, Uden, Holland), CD68 (Dako), neutrophil elastase (Dako), CD3 (Dako) and appropriate isotype controls (Dako). The number of positive nucleated cells was enumerated per mm² of bronchial submucosa, ASM-bundle and mucosal glands.

Statistical analysis

Statistical analysis was performed using prism version 4. Parametric data were expressed as mean (SEM); data that had a log normal distribution was log transformed and described as geometric mean (95% confidence interval); nonparametric data were described as median (IQR). One-way analysis of variance and *t*-tests (Kruskal–Wallis and Mann–Whitney tests for nonparametric data) were used for across and between group comparisons respectively. Chi-squared tests were used to compare categorical data.

Results

Sputum IL-13 concentration in COPD

Clinical and sputum characteristics for subjects in cohort 1 are shown in Table 1. Smoking pack year history was well matched for subjects with disease and controls. The sputum eosinophil count was increased in those subjects with COPD GOLD 3 [3.2 (1.7–12.4)%; $P < 0.001$] and GOLD 4 [3.9 (1.3–11.5)%; $P < 0.001$] compared with that of controls [0.5 (0.3–0.8)%]. Sputum IL-13 was only measurable in 6/34 subjects with COPD and was not detected in any of the healthy controls (Table 1). There was no difference in sputum IL-13 concentration between COPD GOLD 2–4 or healthy controls ($P = 0.3$). In those COPD subjects with measurable vs immeasurable sputum IL-13, there was no difference in sputum eosinophil count [3.5 (1.7–7.2)% vs 2.3 (1.2–4.2)%; $P = 0.6$], total IgE [53 (7) vs 122 (37); $P = 0.47$], smoking history [28 (8) pack years vs 44 (4) pack years; $P = 0.11$] or presence of chronic bronchitis (5/6 vs 18/28; $P = 0.4$).

IL-13 expression in large airway tissue specimens

Examples of IL-13+ cells in airway compartments are shown in Fig. 1. Clinical characteristics of cohort 2 are as shown in Table 2. There was a paucity of IL-13+ cells

within the submucosa, ASM-bundle and glands with no significant differences between groups (Table 2).

Enumeration of inflammatory cell types within compartments in the proximal airway

There were no differences between groups in submucosal or ASM-bundle cellular infiltration (Table 2) although bronchial gland CD3⁺ cells were increased in subjects with COPD compared with healthy controls ($P = 0.04$, Table 2 and Fig. 1). The pattern of inflammatory cells in the different compartments was distinct and the proportion of each cell type per compartment is shown in Fig. 2.

Discussion

In COPD IL-13 expression was not increased in induced sputum or bronchial mucosa. The paucity of IL-13⁺ cells in the bronchial submucosa, glands and ASM-bundle compartments of the airways from patients with GOLD 1–2 COPD was striking and was supported by the very low concentrations of induced sputum IL-13 across disease severity (GOLD 2–4). We identified that inflammatory cells were localized to all of the different airway compartments examined and the pattern of localization was distinct for each inflammatory cell. These patterns were similar between those subjects with and without COPD with the exception of CD3⁺ cells in the bronchial glands which were increased in COPD.

Several lines of evidence support a role for IL-13 in the pathogenesis of COPD. Transgenic mouse models suggest a central role for IL-13 in mucus hypersecretion and emphysema-like peripheral alveolar destruction phenotypes which mimic aspects of COPD (3, 4). BAL lymphocytes from subjects with COPD have increased intracellular IL-13 expression (12) and peripheral blood IL-13 concentration was related to $FEV_1\%$ predicted (13). In chronic bronchitis the number of central airway IL-13⁺ cells was increased compared with asymptomatic smokers (6). In contrast, IL-13 mRNA and protein were decreased in emphysema (5). We report here for the first time in COPD the induced sputum IL-13 concentration and the number of IL-13⁺ cells in different airway compartments from proximal airway specimens. In contrast to our previous findings in asthma (10, 11), we found that IL-13 expression was not increased in COPD. Although we cannot exclude the possibility that IL-13 may be important in a subgroup of subjects with COPD, we were unable to identify clinical features that distinguished those subjects with or without measurable sputum IL-13. Our data therefore challenge the hypothesis of a pathogenic role for IL-13 in COPD.

In addition to examining the expression of IL-13 in proximal airways our study design enabled us to characterize for the first time the inflammatory cell localization to different proximal airway compartments. We found that the number of inflammatory cells was high in the bronchial submucosa, intermediate in the glands and low in the ASM-bundle. The distribution of the inflammatory cells was distinct for each cell type suggesting that recruitment and retention of inflammatory cells to different compartments of the airway wall is under tight control (7). We were unable to demonstrate an increase in the number of neutrophils or T cells in the ASM-bundle as previously reported (14) in the small airways in COPD or an increase in mast cells in the ASM-bundle as observed in asthma (8). Indeed, the

distribution of the inflammatory cells in the airway compartments was remarkably similar for subjects with and without COPD except for the number of T cells in the bronchial glands. We report for the first time that CD3⁺ cells were increased in the bronchial glands in COPD. This is in contrast to previous reports in chronic bronchitis that have suggested increased numbers of mast cells, neutrophils, and macrophages, but not T cells (7, 15). In fatal asthma, the number of mast cells and neutrophils in mucosal glands was associated with mucus plugging (16). It is therefore likely that inflammatory cell-glandular interactions may be important in the development of glandular hyperplasia and mucus hypersecretion, which are important features of COPD.

One potential criticism of our study was its cross-sectional design limited to stable subjects; therefore we cannot exclude the possibility that IL-13 may play a role in COPD exacerbations. We are confident that the sputum IL-13 measurements are robust as we have extensively validated this assay and meaningful differences were observed between subjects with asthma and healthy controls (10, 11). Furthermore the very low concentration of sputum IL-13 across severities reduces the likelihood that this cytokine is important in disease. We confirmed our sputum findings in surgical lung resection specimens. Importantly our study was limited to large airway samples and therefore we cannot exclude the possibility that IL-13 expression may be increased in the smaller airways in COPD. In addition we concede that the control subjects were undergoing surgery and therefore are not true healthy controls, but this would only confound our findings if IL-13 expression was increased in all of the subject groups.

In conclusion, our findings do not support a pathogenic role for IL-13 in COPD. Whether the tissue localization of inflammatory cells to airway compartments in COPD is important and in particular the increase of T cells in glands needs to be further investigated.

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References

1. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med*. 2004; 350:2645–2653. [PubMed: 15215480]
2. Wills-Karp M, Chirramonte M. Interleukin-13 in asthma. *Curr Opin Pulm Med*. 2003; 9:21–27. [PubMed: 12476080]
3. 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; 103:779–788. [PubMed: 10079098]
4. Zheng T, Zhu Z, Wang Z, Homer RJ, Ma B, Riese RJ Jr, et al. Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema. *J Clin Invest*. 2000; 106:1081–1093. [PubMed: 11067861]
5. Boutten A, Bonay M, Laribe S, Leseche G, Castier Y, Lecon-Malas V, et al. Decreased expression of interleukin 13 in human lung emphysema. *Thorax*. 2004; 59:850–854. [PubMed: 15454650]

6. Miotto D, Ruggieri MP, Boschetto P, Cavallero G, Papi A, Bononi I, et al. Interleukin-13 and -4 expression in the central airways of smokers with chronic bronchitis. *Eur Respir J*. 2003; 22:602–608. [PubMed: 14582911]
7. Siddiqui S, Hollins F, Saha S, Brightling CE. Inflammatory cell microlocalisation and airway dysfunction: cause and effect? *Eur Respir J*. 2008; 121:685–691.
8. Brightling NEJM, Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, et al. Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med*. 2002; 346:1699–1705. [PubMed: 12037149]
9. Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. *Am J Respir Crit Care Med*. 2001; 163:1256–1276. [PubMed: 11316667]
10. Berry MA, Parker D, Neale N, Woodman L, Morgan A, Monk P, et al. Sputum and bronchial submucosal IL-13 expression in asthma and eosinophilic bronchitis. *J Allergy Clin Immunol*. 2004; 114:1106–1109. [PubMed: 15536417]
11. Saha SK, Berry M, Parker D, Siddiqui S, Morgan A, May R, et al. Increased sputum and bronchial biopsy interleukin (IL)-13 expression in severe asthma. *J Allergy Clin Immunol*. in press.
12. Barcelo B, Pons J, Fuster A, Sauleda J, Noguera A, Ferrer JM, et al. Intracellular cytokine profile of T lymphocytes in patients with chronic obstructive pulmonary disease. *Clin Exp Immunol*. 2006; 145:474–479. [PubMed: 16907916]
13. Lee JS, Rosengart MR, Kondragunta V, Zhang Y, McMurray J, Branch RA, et al. Inverse association of plasma IL-13 and inflammatory chemokines with lung function impairment in stable COPD: a cross-sectional cohort study. *Respir Res*. 2007; 14:64. [PubMed: 17868461]
14. Baraldo S, Turato G, Badin C, Bazzan E, Beghe B, Zuin R, et al. Neutrophilic infiltration within the airway smooth muscle in patients with COPD. *Thorax*. 2004; 59:308–312. [PubMed: 15047950]
15. Saetta M, Turato G, Facchini FM, Corbino L, Lucchini RE, Casoni G, et al. Inflammatory cells in the bronchial glands of smokers with chronic bronchitis. *Am J Respir Crit Care Med*. 1997; 156:1633–1639. [PubMed: 9372687]
16. Carroll NG, Mutavdzic S, James AL. Increased mast cells and neutrophils in submucosal mucous glands and mucus plugging in patients with asthma. *Thorax*. 2002; 57:677–682. [PubMed: 12149526]

Figure 1.

Example photomicrographs of IL-13+ cells in a chronic obstructive pulmonary disease (COPD) subject in A) submucosa (×400), B) ASM-bundle (×400), C) glands (×400) and D) isotype control (×200). Arrows highlight IL-13+ cells in airway compartments. Additional example photomicrographs of a COPD subject with E) T cells in glands (×400) and F) isotype control (×400).

Figure 2.

Mean (SEM) proportions of inflammatory cells in different airway compartments for all subjects. * $P < 0.05$ comparing the distribution of each cell type across compartments.

Table 1

Cohort 1 subject details, sputum characteristics and sputum IL-13 concentration

	Normal	GOLD 2	GOLD 3	GOLD 4
Number	14	10	14	10
Age	60 (3)	65 (3)	61 (2)	69 (3)
Male (<i>n</i>)	6	5	10	9
Smokers current/ex/ never (<i>n</i>)	5/2/7	4/6/0	4/10/0	1/9/0
Smoking pack years of current and ex smokers	37 (5)	27 (3)	48 (5)	52 (7)
Atopy (<i>n</i>)	6	3	4	5
Total IgE	Not done	47.5 (17.9)	94.9 (44.2)	204.8 (84.4)
Inhaled corticosteroid (µg/day BDP equivalent)	0	0	1650 (123)	1600 (146)
FEV ₁ % predicted	96.2 (2.7)	60.8 (2.0)*	44.1 (6.3)*	23.4 (0.016)*
Bronchodilator response (%)	1.2 (1)	3.0 (1.5)	3.4 (1.4)	3.0 (2.2)
FEV ₁ /FVC %	76.1 (1.7)	60.4 (2.7)*	52.3 (8.7)*	48.3 (3.4)*
Total cell count 10 ⁶ /g sputum [†]	2.7 (1.3–5.4)	3.3 (2.2–4.9)	2.9 (1.9–4.5)	2.7 (1.4–5.9)
Eosinophil (%) [†]	0.5 (0.3–0.8)	1.2 (0.6–3.4)	3.2 (1.7–12.4)*	3.9 (1.3–11.5)*
Neutrophil (%)	56.7 (6.3)	58.9 (9.4)	61.6 (8.4)	68.8 (10.4)
Macrophage (%)	35.7 (6.0)	36.7 (8.9)	27.9 (6.9)	23.1 (9.5)*
Lymphocyte (%)	1.0 (0.23)	0.87 (0.17)	0.45 (0.14)	0.23 (0.091)
Epithelial cells (%)	4.3 (1.76)	1.63 (0.49)	2.99 (1.2)	0.90 (0.31)
Measurable IL-13 (<i>n</i>)	0/14	1/10	3/14	2/10
IL-13 (pg/g), median (range)	0 (0)	0 (0–17.9)	0 (0–64.4)	0 (0–15.2)

Data expressed as mean (SE).

* $P < 0.05$.[†] Geometric mean (lower–upper 95% CI).

BDP-beclomethasone dipropionate.

Table 2

Cohort 2 subject details and inflammatory cell counts in airway structures

	Nonsmokers	Smokers	COPD
Number	8	10	10
Age	55.9 (3.1)	53.3 (7.5)	69.4 (8.0)
Male (<i>n</i>)	6	8	8
Smoking pack years, mean (SEM)	2.5 (2.5)	31.0 (6.6)	47.4 (8.8)
<i>FEV</i> ₁ % predicted, mean (SEM)	88.4 (4.5)	85.6 (3.6)	67.3 (4.0)*
<i>FEV</i> ₁ / <i>FVC</i> (%), mean (SEM)	76.8 (1.9)	76.5 (1.3)	56.0 (2.4)*
Cells/mm ² submucosa			
IL-13 cells	1.3 (2.8)	1.0 (6.3)	0 (0.91)
Mast cells	49.8 (42.8)	38.1 (37.2)	28.6 (42.3)
Eosinophils	0 (0)	0 (4.1)	0 (11.1)
Neutrophils	41.5 (25.1)	28.1 (29.2)	22.4 (83.9)
Macrophages	47.0 (42.9)	87.9 (83.5)	25.7 (44.2)
T cells	36.2 (72.2)	26 (51.7)	24.8 (55.7)
Cells/mm ² ASM-bundle			
IL-13 cells	0 (0.02)	0 (0.13)	0 (0)
Mast cells	7.9 (5.9)	7.3 (15.2)	5.0 (12.5)
Eosinophils	0 (0)	0 (0)	0 (0)
Neutrophils	0 (0.45)	0 (0.78)	0.25 (4.22)
Macrophages	10.6 (11.7)	6.2 (9.9)	3.9 (13.1)
T cells	1.3 (4.8)	2.2 (7.3)	2.7 (4.0)
Cells/mm ² glands			
IL-13 cells	0 (0.35)	0.16 (0.97)	0 (0.35)
Mast cells	12.9 (12.5)	18.7 (18.5)	21.9 (27.0)
Eosinophils	6.2 (9.5)	2.9 (6.6)	7.8 (13.3)
Neutrophils	8.0 (14.3)	4.2 (9.5)	4.9 (8.63)
Macrophages	7.3 (28.3)	6.5 (11.2)	11.1 (36.1)
T cells	2.8 (10.1)	2.8 (50.9)	36.4 (100.8)*

* $P < 0.05$.

All data expressed are median (IQR).

ASM, airway smooth muscle.