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

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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 ($R_s = 0.4$, $P \leq .05$).

Conclusions: IL-13 overexpression in sputum and bronchial biopsy specimens is a feature of severe asthma. (J Allergy Clin Immunol 2008;121:685-91.)

Key words: *Severe asthma, IL-13, sputum, bronchus, airway smooth muscle, eosinophilia*

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 corticosteroid-naive 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-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

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Abbreviations used
ACQ: Juniper Asthma Control Questionnaire
AHR: Airway hyperresponsiveness
ASM: Airway smooth muscle
GINA: 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 MINITAB13.31 (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 $IL\text{-}13^+$ cells in the bronchial submucosa and ASM bundle are shown in Table III. Representative photomicrographs of $IL\text{-}13^+$ 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 $IL\text{-}13^+$ 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 $IL\text{-}13^+$ 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 $IL\text{-}13^+$ 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 $IL\text{-}13^+$ cells in the ASM bundle was increased in the subjects with severe