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Title

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Journal

American Journal of Respiratory and Critical Care Medicine, 190(6)

ISSN

1073-449X

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Publication Date

2014-09-15

DOI

10.1164/rccm.201403-0505oc

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Epithelial Interleukin-25 Is a Key Mediator in Th2-High, Corticosteroid-Responsive Asthma

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Abstract

Rationale: Activation of type 2 cytokine pathways plays a central role in a large subset of subjects with asthma. Th2-high and Th2-low asthma have distinct clinical, pathologic, and molecular phenotypes and respond differently to therapy. The factors that initiate type 2 responses in some subjects with asthma are unknown.

Objectives: To determine whether expression of epithelial cytokines IL-25, IL-33, and thymic stromal lymphopoietin are associated with type 2 responses and predict response to inhaled corticosteroid (ICS) in asthma.

Methods: We analyzed pulmonary function tests, blood, and bronchoscopic biopsies from 21 healthy control subjects and 43 subjects with asthma. Subjects with asthma underwent an 8-week treatment with inhaled budesonide.

Measurements and Main Results: Epithelial expression of IL-25, but not IL-33 or thymic stromal lymphopoietin, was increased in a subset of subjects with asthma. The IL-25-high subset had greater airway hyperresponsiveness, more airway and blood eosinophils, higher serum IgE, more subepithelial thickening, and higher expression of Th2 signature genes. ICS improved FEV_1 and hyperresponsiveness in the IL-25-high but not the IL-25-low subset. Plasma IL-25 levels correlated with epithelial IL-25 expression, airway eosinophilia, and beneficial responses to ICS treatment.

Conclusions: IL-25 measurements identify two subsets of subjects with distinct asthma phenotypes and different responses to ICS. Because IL-25 has a major role in triggering type 2 responses, bronchial epithelial IL-25 expression is likely a key determinant of type 2 response activation in asthma. Plasma IL-25 level reflects airway IL-25/type 2 response activation and may be useful for predicting responses to asthma therapy.

Keywords: IL-25; asthma; phenotype

Asthma is a heterogeneous disease that can be subclassified based on clinical, pathologic, and molecular features (1–3). Clinical phenotyping can be based on asthma severity and response to therapy (1). Pathologic phenotyping can be based on the type of inflammatory cells in airway biopsies or induced sputum (2, 4). Molecular phenotyping (or endotyping) can be based on the expression of cytokines in airway tissues (3) or induced sputum (5, 6). One major asthma endotype is Th2-high

(Received in original form March 18, 2014; accepted in final form August 11, 2014)

*These authors made equal contributions.

Supported by National Natural Science Foundation of China (grants 81170022 and 30700244), Scientific Research Foundation for the Returned Overseas Chinese Scholars of State Education Ministry grant (2008890), National Key Technology R&D Program of the 12th Five-year Development Plan (2012BAI05B01), the UCSF Sandler Asthma Basic Research Center, and the National Institutes of Health (grants AI1077439 and HL099101).

Author Contribution: G.Z. designed and conducted the clinical study, sample analyses, statistical analyses, conceived of the manuscript, and had primary responsibility for writing. D.C., Z.X., L.Y., H.S., K.Z., and X.H. performed sample collection and sample analyses. L.R.B. assisted with immunofluorescent staining. J.Z. and Y.X. contributed to clinical study design. D.J.E. assisted with statistical analyses and writing of the manuscript. All authors reviewed and approved the manuscript.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 190, Iss 6, pp 639–648, Sep 15, 2014
Copyright © 2014 by the American Thoracic Society
Originally Published in Press as DOI: 10.1164/rccm.201403-0505OC on August 18, 2014
Internet address: www.atsjournals.org

> **At a Glance Commentary**
>
> **Scientific Knowledge on the Subject:** Type 2 responses play prominent roles in a subset of individuals with asthma. Epithelial cytokines can trigger type 2 responses but the contributions of specific epithelial cytokines to Th2-high asthma have not been well defined.
>
> **What This Study Adds to the Field:** Our results strongly implicate epithelial IL-25 as a major driver of Th2-high, inhaled corticosteroid–responsive asthma. Plasma IL-25 level reflects airway IL-25/type 2 response activation and may be useful for predicting responses to asthma therapy.

asthma. Type 2 cytokines produced by airway $CD4^+$ T cells (Th2 cells) and by type 2 innate lymphoid cells (iLC2s) can act on resident airway cells, including epithelial and smooth muscle cells, to produce key features of asthma, including eosinophilic inflammation, airway hyperresponsiveness, and mucus overproduction. The type 2 cytokine IL-13 is necessary and sufficient for each of these abnormalities in many animal models (7, 8). Human studies indicate that IL-13 is increased in a subset of subjects with asthma, and that these Th2-high subjects have different clinical and pathologic characteristics than Th2-low subjects with asthma (3). For example, Th2-high subjects had improvements in FEV_1 following inhaled corticosteroid (ICS) treatment, whereas Th2-low subjects did not. However, the mechanisms that activate the type 2 responses in subjects with Th2-high asthma are largely unknown.

Airway epithelial cells are important for allergen uptake, sensitization to allergen, and initiation of allergic inflammation (9). Epithelial-derived cytokines can trigger type 2 responses by multiple mechanisms. The epithelial cytokine thymic stromal lymphopoietin (TSLP) conditions dendritic cells and also acts directly on naive $CD4^+$ T cells to promote Th2 cell differentiation (10, 11). The epithelial cytokines IL-25 and IL-33 act on multiple immune cells, including mast cells, basophils, and iLC2s (12–17). IL-25 (18–21), IL-33 (22), and TSLP (11) have each been reported to be both necessary and sufficient for type 2 cytokine production, eosinophilic airway inflammation, mucous metaplasia, and airway hyperresponsiveness in certain mouse models. In human studies, bronchial expression of TSLP (23), IL-25 (24, 25), and IL-33 (26) have been reported to be increased in asthma. However, it is not yet known whether differences in expression of one or more of these epithelial cytokines are associated with Th2-high/Th2-low status and response to asthma therapy.

We hypothesized that the epithelial cytokines IL-25, IL-33, and TSLP are expressed at different levels in subjects with asthma and that these differences are associated with the clinical, pathologic, and molecular heterogeneity. To test our hypothesis, we recruited 21 healthy control subjects and 43 subjects with asthma and examined epithelial cytokine expression. After determining that IL-25 expression was elevated in a subset of subjects with asthma, we compared the clinical, pathologic, and molecular phenotypes of IL-25-high and IL-25-low asthma. Because epithelial IL-25 expression is difficult to measure, we also investigated whether measurements of plasma IL-25 could be used as a surrogate.

Methods

Subjects

We recruited 21 healthy control subjects and 43 subjects with asthma. All subjects were Chinese and were recruited from Tongji Hospital. Subjects with asthma were diagnosed by a physician; had symptoms of episodic cough, wheeze, and/or breathlessness; and had accumulated dosage of methacholine provoking a 20% fall in FEV_1 (PD_{20}) less than 2.5 mg and/or greater than or equal to 12% increase in FEV_1 following inhalation of 200 μg salbutamol. Physicians referred the subjects with asthma to the study personnel after they established a new diagnosis of asthma and decided to initiate the standard ICS regimen in use at Tongji Hospital. After baseline evaluation by study personnel, subjects initiated ICS prescribed by the referring physician. Healthy control subjects had no respiratory symptoms, normal spirometric value, and methacholine PD_{20} greater than 2.5 mg. None of the subjects had ever smoked or received ICS or oral corticosteroid or leukotriene antagonist. Written informed consent was obtained from all subjects. The ethics committee of Tongji Hospital, Huazhong University of Science and Technology, approved the study.

Baseline Evaluation

For each subject, at study entry we recorded demographic information, collected blood and induced sputum samples, and performed spirometry and allergen skin prick testing with a panel of 14 aeroallergens. We performed bronchoscopy with brushing and endobronchial biopsies within 1 week of study entry. Biopsy techniques and methods for histology, immunohistochemistry, and gene expression analysis by quantitative polymerase chain reaction are detailed in the METHODS section of the online supplement.

ICS Treatment

All subjects with asthma were placed on a standard ICS regimen (budesonide,

Table 1. Overall Subject Characteristics*

	Normal Control Subjects	Subjects with Asthma	*P* Value
Number	21	43	—
Age, yr	35 ± 10	35 ± 10	0.75
Sex, M:F (% F)	9:12 (57)	17:26 (60)	0.80
FEV_1, % predicted	94 (90.1–101.9)	85 (72.3–91.2)	<0.0001
Methacholine PD_{20}, mg	4.7 (3.5–5.5)	0.02 (0.01–0.14)	<0.0001
IgE, IU/ml	20.5 (8.6–46.7)	141 (39.6–289.2)	<0.0001
Blood eosinophils, $\times 10^9$/L	0.07 ± 0.07	0.38 ± 0.21	<0.0001

Definition of abbreviation: PD_{20} = provocative dosage required to cause a 20% decline in FEV_1.
*Values are presented as mean ± SD or median (interquartile range). *P* values are Sidak corrected for multiple testing (across the three groups).

200 μg twice a day) by their personal physicians. Subjects began using ICS immediately after the bronchoscopy procedure and continued to use ICS throughout the 8-week study period. All subjects underwent pulmonary function testing at 4 and 8 weeks and had blood samples collected at 4 weeks.

Statistical Analysis

We analyzed data using Prism version 5 (GraphPad Software, San Diego, CA) and SPSS version 19 (SPSS Inc., Chicago, IL).

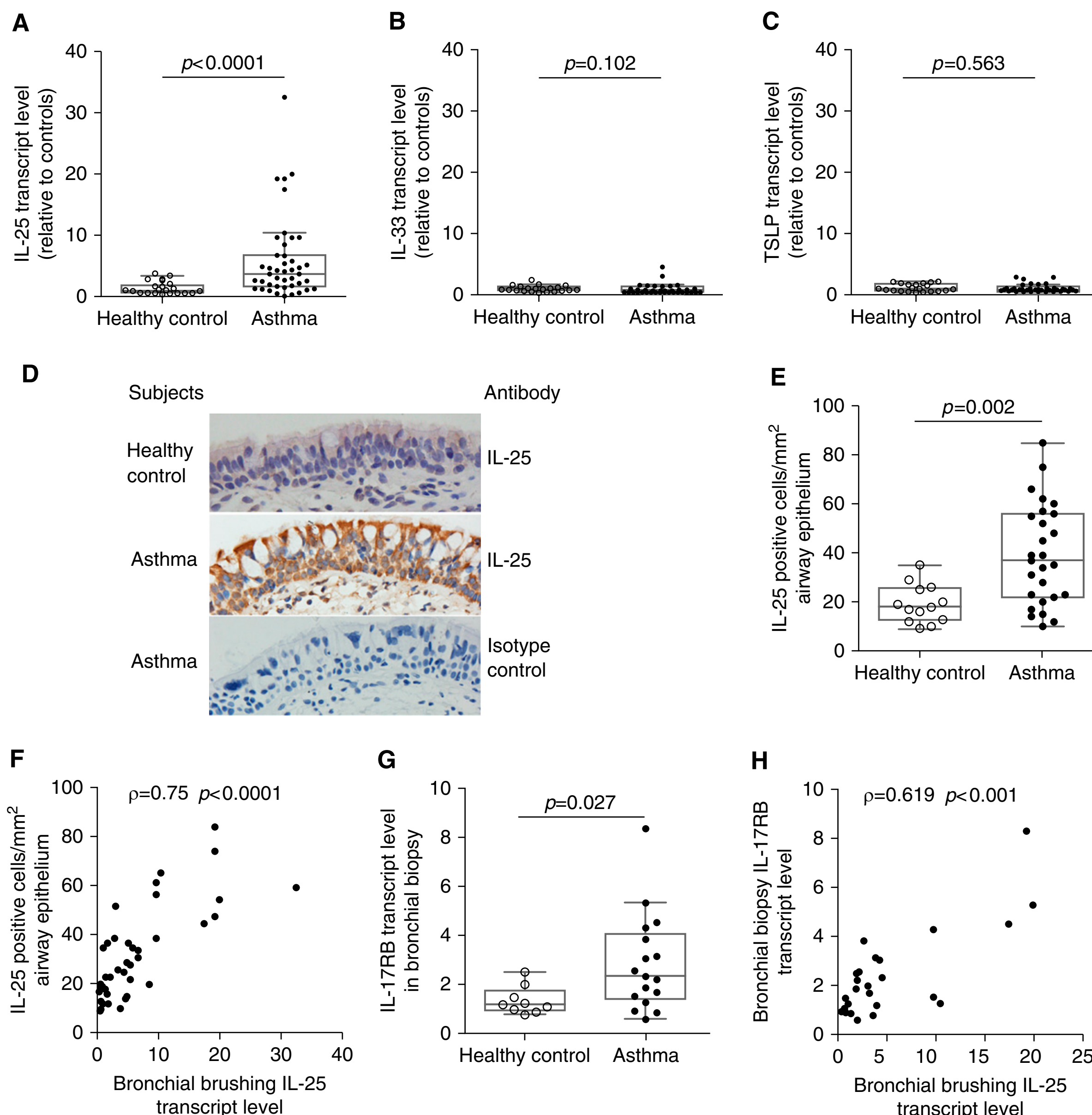

Figure 1. Bronchial epithelial expression of IL-25 and its receptor are increased in some subjects with asthma. Levels of IL-25 (*A*), IL-33 (*B*), and thymic stromal lymphopoietin (TSLP) (*C*) transcripts were determined by quantitative polymerase chain reaction (PCR) of RNA from bronchial brushings of healthy control subjects (n = 21) and subjects with asthma (n = 43). Values are relative to the median value for healthy control subjects. The median IL-25 cycle threshold values for healthy control subjects and asthma subjects were 24.06 and 22.14, respectively. (*D*) IL-25 immunohistochemistry in bronchial biopsies from healthy control subjects and subjects with asthma (original magnification ×400). (*E*) Quantitation of IL-25–containing cells in the epithelium of healthy control subjects (n = 13) and subjects with asthma (n = 27). (*F*) Correlation between bronchial brushing IL-25 transcript level and the number of IL-25–containing cells in airway epithelium for healthy control subjects (n = 13) and subjects with asthma (n = 27). (*G*) The transcript level of IL-25 receptor, IL-17RB, as determined by quantitative PCR of RNA from bronchial biopsies of healthy control subjects (n = 9) and subjects with asthma (n = 17). (*H*) Correlation between bronchial brushing IL-25 transcript level and IL-17RB transcript level in bronchial biopsies of healthy control subjects (n = 9) and subjects with asthma (n = 17). Some subjects were not included in the analyses of IL-25 protein and/or IL-17RB transcript level because bronchial biopsies from those subjects were not adequate for reverse transcriptase PCR and/or immunohistochemistry because of limitations in the number of biopsies obtained or the amount of intact tissue seen in the sections.

Table 2. Subject Characteristics by IL-25 Expression*

	Normal Control Subjects	Subjects with Asthma		P Value (Low vs. High)
		IL-25 Low	IL-25 High	
Number	21	22	21	—
Age, yr	35 ± 10	37 ± 10	33 ± 11	0.23
Sex, M:F (% F)	9:12 (57)	9:13 (59)	8:13 (62)	0.85
Body mass index	21.8 ± 3.0	22.5 ± 3.7	21.6 ± 3.2	0.38
Age at onset	—	32 ± 10	31 ± 11	0.68
Duration of asthma	—	3 ± 6	3 ± 3	0.58
FEV_1, % predicted	94 (90.1–101.9)	85.2 (76.9–94.1)	84.8 (69.9–90.3)	0.65
Methacholine PD_{20}, mg	3.9 (3–5.1)	0.04 (0.02–0.33)	0.01 (0.01–0.05)	0.048
IgE, IU/ml	20.5 (8.6–46.7)	63.6 (24.4–152.3)	219.4 (97.8–683)	0.001
Blood eosinophils, $\times 10^9$/L	0.07 ± 0.07	0.31 ± 0.2	0.45 ± 0.21	0.045
Sputum eosinophils, %	0.13 ± 0.22	6.34 ± 8.53	14.68 ± 13.62	0.009
Biopsy eosinophils, number/mm^2	0 (0–0.8); n = 15	7.8 (4.3–15); n = 18	23.7 (21–28); n = 15	0.0003
Atopic, %	2 (10)	22 (100)	21 (100)	—
Basement membrane thickness, μm	5.19 (4.9–5.7); n = 15	6.2 (5.43–8.5); n = 18	10.3 (8.4–11.1); n = 15	0.0006
ΔFEV_1 with ICS at 4 wk, L	—	0.12 ± 0.05	0.35 ± 0.11	0.001
ΔFEV_1 with ICS at 8 wk, L	—	0.11 ± 0.09	0.34 ± 0.10	0.001

Definition of abbreviations: ICS = inhaled corticosteroids; PD_{20} = provocative dosage required to cause a 20% decline in FEV_1.
*Values are presented as mean ± SD or median (interquartile range) unless otherwise specified. *P* values are Sidak corrected for multiple testing (across the three groups). In case of missing data, the number of subjects for whom data exist is noted.

For normally distributed data we calculated means ± SD and we used parametric tests (one-way analysis of variance with Tukey correction or unpaired *t* test) to compare across groups. For nonnormally distributed data we calculated medians (with interquartile ranges) and used nonparametric tests (Kruskal-Wallis test with Dunn intergroup comparison or Mann-Whitney test). We analyzed correlation using Spearman rank order correlation. For significance testing of PD_{20} and serum IgE levels, data were log transformed for normality.

Results

Subject Characteristics

Subject characteristics are summarized in Table 1. Subjects with asthma had lower FEV_1 and methacholine PD_{20} and higher serum IgE levels and blood eosinophil numbers than healthy control subjects.

Airway Epithelial IL-25, IL-33, and TSLP Expression

We measured the levels of IL-25, IL-33, and TSLP transcripts in bronchial brushing samples by quantitative polymerase chain reaction. IL-25 transcript levels in bronchial brushing samples of subjects with asthma were significantly higher than in healthy control subjects (Figure 1A). IL-25 expression was heterogeneous: some subjects with asthma had IL-25 levels that were within the normal range, whereas others had levels that were substantially above the normal range. There were no significant differences in IL-33 and TSLP transcript levels between subjects with asthma and healthy control subjects (Figures 1B and 1C).

IL-25 immunostaining revealed that IL-25–containing cells were mainly airway epithelial cells. Consistent with our analysis of IL-25 transcript levels, only some subjects with asthma had increased numbers of IL-25–containing cells compared with healthy control subjects (Figures 1D and 1E). Moreover, the number of IL-25–containing cells was correlated with IL-25 transcript levels in bronchial brushing samples (Figure 1F). We also examined the expression of the IL-25 receptor, IL-17RB, which is known to be induced by IL-25 (24). IL-17RB transcript levels in bronchial biopsies of subjects with asthma were significantly higher than in healthy control subjects (Figure 1G). Some subjects with asthma had IL-17RB transcript levels within the normal range, whereas others had levels above the normal range. IL-17RB transcript levels in biopsies correlated with IL-25 levels in bronchial brushings from the same subjects (Figure 1H). We detected IL-17RB protein in bronchial epithelial cells and some subepithelial cells by immunohistochemistry (*see* Figure E1A in the online supplement). The number of IL-17RB–staining epithelial cells was higher in subjects with asthma than

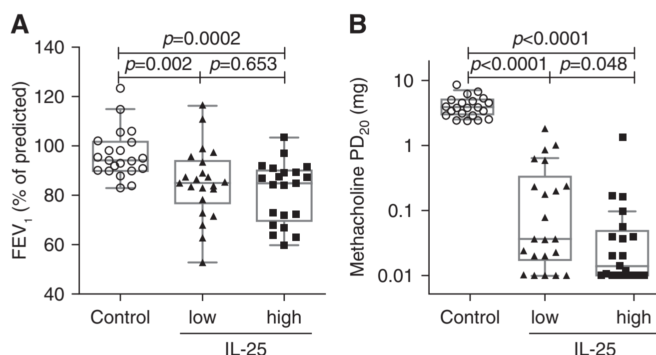

Figure 2. Pulmonary function test results in IL-25-low and IL-25-high asthma. (*A*) FEV_1, a measure of airway obstruction. (*B*) Methacholine-provocative dosage required to cause a 20% decline in FEV_1 (PD_{20}), a measure of airway hyperresponsiveness.

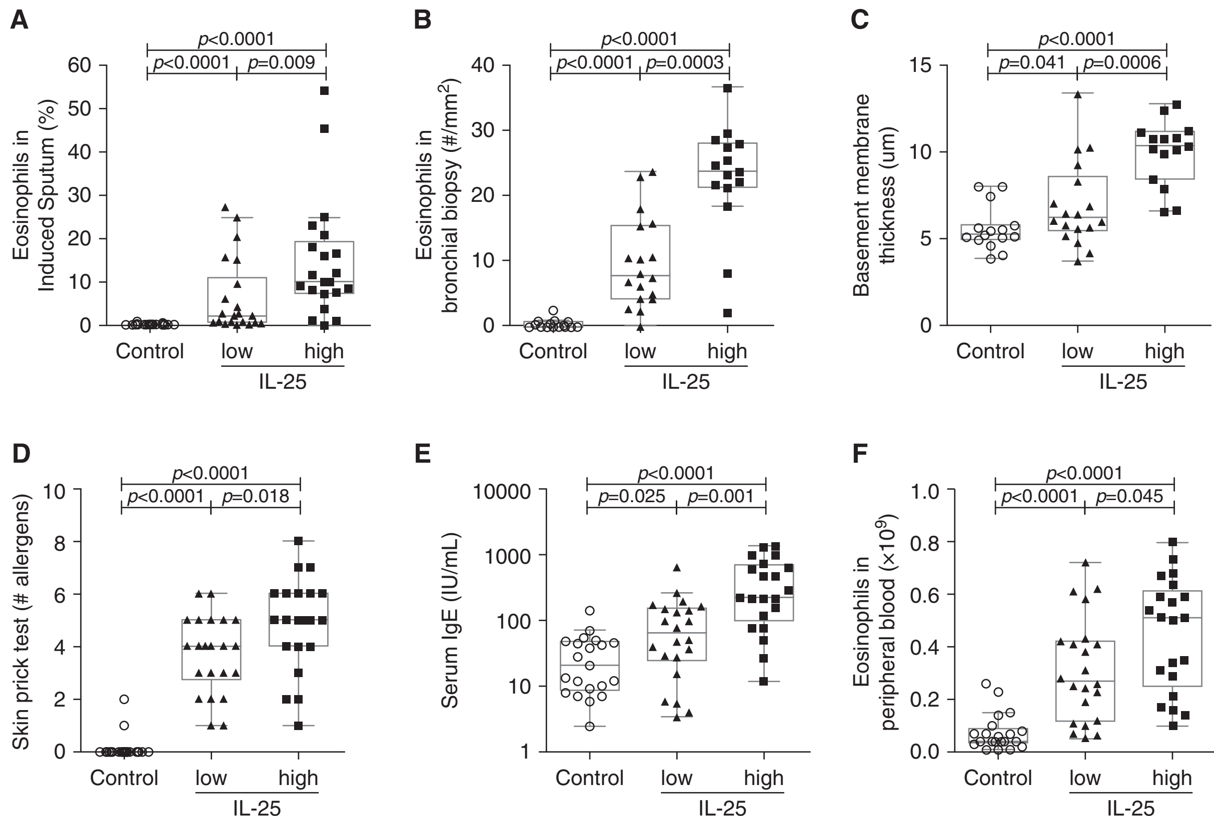

Figure 3. Measures of airway eosinophilic inflammation, airway remodeling, and allergy are increased in subjects with IL-25-high asthma. (*A*) Eosinophils as a percentage of total cells in induced sputum. (*B*) The number of eosinophils per square millimeter of submucosa in bronchial biopsy. (*C*) Reticular basement membrane thickness, a measure of subepithelial fibrosis. (*D*) Skin prick test using a panel of 14 aeroallergens. (*E*) Serum IgE. (*F*) Peripheral blood eosinophil count.

in healthy control subjects and the number of IL-17RB–positive cells in airway epithelium was significantly correlated with the number of IL-25–positive cells (*see* Figures E1B and E1C). Our data indicate that the activation of IL-25 pathway is limited to a subset of subjects with asthma.

Clinical Characteristics of IL-25-Low and IL-25-High Asthma

Our finding that the IL-25 pathway was only activated in a subset of subjects with asthma prompted us to hypothesize that the IL-25 pathway is involved in asthma clinical heterogeneity. We classified the subjects with asthma into IL-25-high and IL-25-low subgroups according to IL-25 transcript levels in bronchial brushing samples. We classified subjects with asthma as IL-25-low (at or below the 95th percentile for healthy control subjects) and IL-25-high (above the 95th percentile for healthy control

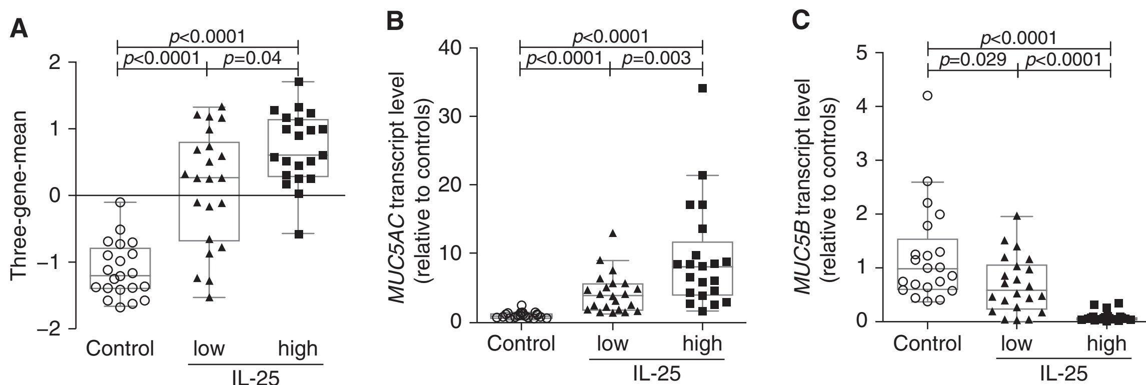

Figure 4. Bronchial epithelial IL-25 transcript levels are associated with the epithelial expression of Th2 signature genes and mucins. (*A*) The three-gene-mean derived from the transcript levels of Th2 signature gene *CLCA1*, *POSTN*, and *SERPINB2* as determined by quantitative polymerase chain reaction in bronchial brushings. (*B*, *C*) Levels of transcripts for the major airway mucins MUC5AC (*B*) and MUC5B (*C*) in bronchial brushings as determined by quantitative polymerase chain reaction. Values are relative to the median value for healthy control subjects.

subjects). According to this classification, 21 of 43 subjects with asthma were IL-25-high. Subjects in these two groups had similar demographic characteristics and FEV_1 (Table 2 and Figure 2A). However, IL-25-high subjects with asthma had more severe airway hyperresponsiveness (as measured by methacholine PD_{20}) (Figure 2B).

Pathologic Characteristics and Markers of Allergic Sensitization in IL-25-Low and IL-25-High Asthma

Eosinophilic airway inflammation and airway remodeling are common features of asthma and IL-25 can induce these abnormalities in animal models (18, 27). We found that IL-25-high subjects with asthma had more marked eosinophilic airway inflammation, as reflected by higher levels of eosinophils in both sputum and airway submucosa from airway biopsies (Figures 3A and 3B). To investigate whether IL-25 expression was associated with airway remodeling, we measured basement membrane thickness, a measure of subepithelial fibrosis, in bronchial biopsies. IL-25-high subjects with asthma had greater basement membrane thickness than IL-25-low subjects with asthma (Figure 3C). These results indicate that epithelial IL-25 expression is associated with more severe eosinophilic airway inflammation and airway remodeling in subjects with asthma.

Skin prick tests, serum IgE level, and peripheral blood eosinophil counts are measures of allergic status. IL-25-high subjects with asthma had more positive skin tests than IL-25-low subjects with asthma (Figure 3D). We did not find any apparent relationship between IL-25 status and responses to specific allergens (*see* Figure E2). IL-25-high subjects also had higher serum IgE levels and peripheral blood eosinophil counts (Figures 3E and 3F).

Airway Epithelial Expression of Th2 Signature Genes and Mucins in IL-25-Low and IL-25-High Asthma

Previous studies indicate that epithelial expression of IL-13–induced genes can be used to classify Th2-high and Th2-low subjects with asthma (3, 5). To determine whether epithelial IL-25 expression is associated with this measure of Th2 status, we examined the expression of the IL-13–induced genes *CLCA1*, *POSTN*, and *SERPINB2* in bronchial epithelial brushings by quantitative polymerase chain reaction. We combined these measurements to calculate a three-gene-mean for each subject as described previously (28). The three-gene-mean was higher in IL-25-high as compared with IL-25-low subjects with asthma (Figure 4A). Levels of periostin mRNA (*POSTN*), a potential biomarker for eosinophilic airway inflammation (29), were significantly higher in IL-25-high as compared with IL-25-low subjects with asthma (*see* Figure E3). Our data indicate that the IL-25 pathway is associated with more prominent epithelial type 2 responses in subjects with asthma.

Mucus overproduction is another essential feature of asthma. MUC5AC and MUC5B are the major gel-forming mucins in human airway mucus (30). Both mucins are regulated by IL-13, and previous work showed that Th2-high asthma is characterized by elevated levels of MUC5AC mRNA and reduced levels of MUC5B compared with Th2-low asthma or healthy control subjects (3). We found that MUC5AC transcript levels in IL-25-high and IL-25-low subjects with asthma were significantly higher than in healthy control subjects. Moreover, IL-25-high subjects with asthma had significantly higher MUC5AC transcript levels than IL-25-low subjects with asthma (Figure 4B). In contrast, MUC5B transcript levels were significantly lower in subjects with asthma when compared with healthy control subjects, and were even lower in IL-25-high as compared with IL-25-low subjects with asthma (Figure 4C). Immunofluorescent staining for MUC5AC and MUC5B proteins in a limited number of biopsies was consistent with the differences in mucin expression detected by mRNA analysis (*see* Figure E4).

Distinct Responsiveness to ICS in IL-25-Low and IL-25-High Asthma

To determine whether airway epithelial IL-25 expression is associated with ICS responsiveness in subjects with asthma, we measured FEV_1 and PD_{20} after 4 and 8 weeks of treatment with inhaled

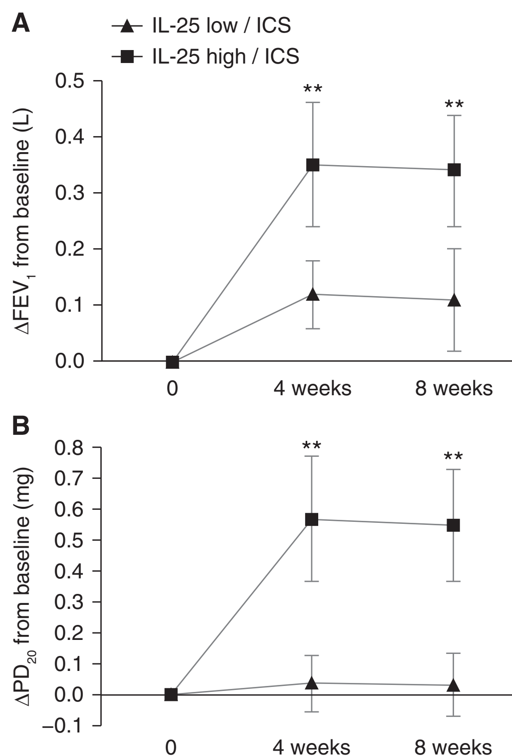

Figure 5. Subjects with IL-25-high asthma have larger improvements in pulmonary function during inhaled corticosteroid (ICS) treatment. Twenty-two IL-25-low subjects and 21 IL-25-high subjects (as determined by epithelial IL-25 mRNA level) with asthma all received ICS treatment. FEV_1 (*A*) and provocative dosage required to cause a 20% decline in FEV_1 (PD_{20}) (*B*) were measured at baseline (0) and after 4 or 8 weeks on daily inhaled budesonide (200 μg twice a day). $^{**}P < 0.01$ for IL-25-low/ICS versus IL-25-high/ICS.

budesonide. Subjects with IL-25-high asthma had significantly more improvement in FEV_1 during treatment with inhaled budesonide when compared with ICS-treated subjects with IL-25-low asthma (Figure 5A). During ICS treatment, PD_{20} improved in subjects with IL-25-high asthma but not in subjects with IL-25-low asthma (Figure 5B). These data indicate that the IL-25 pathway is involved in the heterogeneity of ICS responsiveness in asthma.

Plasma IL-25 Levels Are Associated with Epithelial IL-25 Expression and Eosinophilic Airway Inflammation

Asthma phenotyping based on bronchoscopic samples has limited application. Therefore, we examined IL-25 levels in peripheral blood by ELISA. Consistent with our findings in bronchial epithelial brushings, plasma IL-25 levels were only increased in some subjects with asthma. Subjects classified as IL-25-high based on epithelial IL-25 mRNA had significantly higher plasma IL-25 levels than subjects classified as IL-25-low (Figure 6A). Plasma IL-25 levels were correlated with the expression of IL-25 in bronchial epithelial brushings (Figure 6B). This indicates that plasma IL-25 may be a useful surrogate for airway epithelial IL-25 expression.

We examined the relationship between plasma IL-25 and eosinophilic airway inflammation. We used a previously described cutoff to classify the sputum eosinophil status (3% eosinophils of total sputum inflammatory cells) and bronchial eosinophil status (22 eosinophils per square millimeter submucosa) (31, 32). We found a strong association between airway eosinophilia and plasma IL-25 levels in steroid-naive asthma (Figures 6C and 6D).

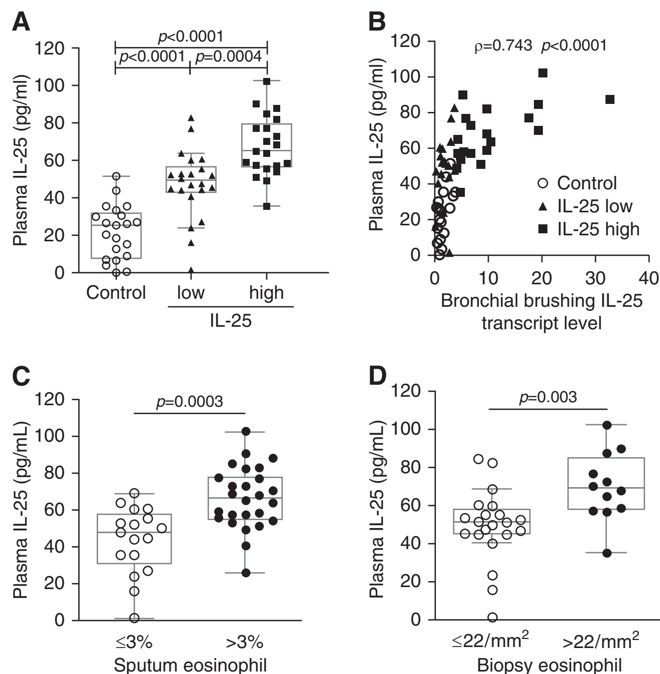

Figure 6. Increased plasma IL-25 levels are associated with increased bronchial epithelial IL-25 expression and airway eosinophilia. (*A*) Plasma IL-25 level in peripheral blood as determined by ELISA. (*B*) Correlation between bronchial brushing IL-25 transcript levels and plasma IL-25 levels of all subjects, including healthy control subjects and subjects with asthma. (*C*) Plasma IL-25 levels in subjects with asthma with and without sputum eosinophilia. (*D*) Plasma levels in subjects with asthma and without submucosal eosinophilia in bronchial biopsies.

Plasma IL-25 Level Is Associated with the ICS Response of Subjects with Asthma

We further explored the relationship between plasma IL-25 level and ICS response of subjects with asthma. We selected a plasma IL-25 threshold of 55 pg/ml to maximize the concordance with IL-25 status based on epithelial IL-25 mRNA levels (*see* Table E2). Improvement of FEV_1 and PD_{20} was significantly greater in subjects with high plasma IL-25 (>55 pg/ml) compared with those with low plasma IL-25 (≤55 pg/ml) after 4 and 8 weeks of inhaled budesonide treatment (Figures 7A and 7B). These data indicate that plasma IL-25 level is associated with ICS responses of subjects with asthma. To evaluate the utility of plasma IL-25 as a predictor of ICS responsiveness (defined as >7.5% improvement in FEV_1 after 8 weeks of ICS [33]), we performed receiver operating characteristic analysis (*see* Figure E5). Area under the curve for plasma IL-25 compared favorably with areas under the curve for blood eosinophil number and sputum eosinophil percentage, and was slightly lower than the area under the curve obtained using biopsy eosinophil number, which requires an invasive procedure.

We also examined plasma IL-25 level in subjects with asthma after ICS treatment. We found that plasma IL-25 was significantly decreased after treatment with inhaled budesonide for 4 weeks. The decrease of plasma IL-25 was mainly observed in IL-25-high subjects. There was no significant change of plasma IL-25 in IL-25-low subjects (*see* Figure E6). This suggests that ICS treatment inhibits airway IL-25 expression in IL-25-high asthma.

Discussion

We provide a new method for molecular phenotyping (endotyping) of asthma based on expression of the airway epithelial cytokine IL-25. We found that IL-25-high and IL-25-low subjects with asthma had distinct clinical and pathologic characteristics and distinct responses to ICS. Airway epithelial IL-25 expression correlated with Th2 signature gene expression. These results suggest that IL-25 expression is a major determinant of type 2 status (Th2-high vs. Th2-low) in asthma. Another notable finding was that plasma IL-25 levels correlated with airway epithelial IL-25 expression and were associated with ICS response in subjects with asthma. Our results indicate that measurements of plasma IL-25 are useful for asthma phenotyping and suggest that IL-25 measurements may be valuable for selecting appropriate asthma treatments.

We measured the airway epithelial expression of epithelial cytokines IL-25, IL-33, and TSLP. Each of these cytokines was reported to be elevated in prior studies of asthma (23, 24, 26). Although our study

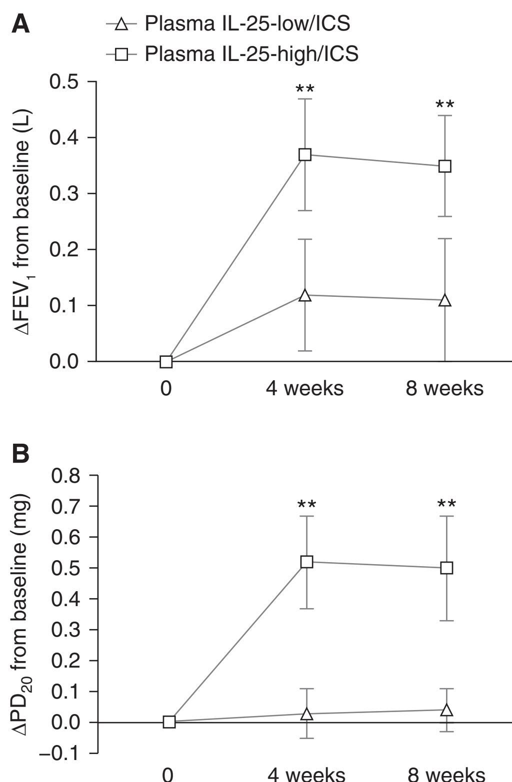

Figure 7. Subjects with elevated plasma IL-25 have larger improvements in pulmonary function during inhaled corticosteroid (ICS) treatment. Twenty plasma IL-25-low subjects (≤55 pg/ml) and 23 plasma IL-25-high subjects (>55 pg/ml) with asthma all received ICS treatment. FEV_1 (*A*) and provocative dosage required to cause a 20% decline in FEV_1 (PD_{20}) (*B*) were measured at baseline (0) and after 4 or 8 weeks on daily inhaled budesonide (200 μg twice a day). $^{**}P < 0.01$ for plasma IL-25-low/ICS versus plasma IL-25-high/ICS.

was larger than previous studies that showed increases in IL-33 (26) and TSLP (23) mRNAs in asthma, we did not detect changes in these mRNAs in our study. This likely reflects differences in study populations and not a technical limitation of our approach, because the method we used for measuring cytokine expression is highly quantitative and readily detected differences in expression of other genes. Although we did not detect differences in IL-33 and TSLP mRNAs, our data do not exclude the possibility that post-transcriptional regulation of these cytokines contributes to asthma heterogeneity in our subjects. Unlike IL-33 and TSLP, IL-25 was elevated in our group of subjects with asthma. Although there was a significant increase in IL-25 in the group of subjects with asthma, many subjects in the group had normal IL-25 levels. This is consistent with a recent report that IL-25 transcript level was increased in induced sputum from some individuals with asthma (34). None of our subjects were using oral corticosteroids or ICS or leukotriene antagonist before enrollment, which excludes the possibility that prior antiinflammatory medications accounted for differences in IL-25 expression. In summary, airway epithelial IL-25 mRNA was increased in some subjects with asthma, whereas we did not detect differences in IL-33 and TSLP mRNA levels between healthy control subjects and subjects with asthma.

IL-25 has been reported to induce iLC2s and $CD4^+$ T cells to produce IL-4, IL-5, and IL-13 and to play a critical role in airway eosinophilia, mucus overproduction, and airway remodeling in mouse models of allergic airway disease (20, 35). To assess the relationship between IL-25 expression and asthma phenotypes, we classified subjects with asthma based on epithelial IL-25 expression and compared clinical, pathologic, and molecular characteristics of the IL-25-high and IL-25-low subgroups. Although a previous study (24) showed that the number of IL-25-immunoreactive cells in airway biopsies correlated inversely with FEV_1, we found no significant correlation between epithelial IL-25 expression and FEV_1 in our larger cohort. However, we did find that subjects with IL-25-high asthma had more marked airway hyperresponsiveness, airway and blood eosinophilia, IgE elevations, skin test reactivity, and subepithelial layer thickening than either IL-25-low subjects with asthma or healthy control subjects. Furthermore, hyperresponsiveness and lung function (as measured by FEV_1) improved during ICS treatment in IL-25-high subjects with asthma but not in IL-25-low subjects. These differences are reminiscent of differences that have been described when comparing Th2-high and Th2-low asthma (3). To directly address whether differences in IL-25 status are associated with differences in type 2 status, we used the validated three-gene-mean metric (28), which incorporates three IL-13 responsive epithelial genes. This analysis confirmed that IL-25 status was strongly associated with type 2 status. In addition to the three genes included in the three-gene metric, we also saw differences in expression of IL-13–regulated mucin genes. *MUC5AC*, which is selectively increased in Th2-high asthma (3), was also increased in IL-25-high subjects and *MUC5B*, which is decreased in Th2-high asthma, was also decreased in IL-25-high subjects. These results demonstrate a strong association between airway type 2 responses and elevated epithelial IL-25 expression in our population. Taken together with the extensive body of evidence that shows that IL-25 initiates type 2 responses and causes eosinophilic inflammation, subepithelial fibrosis, and airway hyperresponsiveness in mouse airway disease models (18–20, 27), our results strongly suggest that increases in human airway epithelial IL-25 expression are a major contributor to airway type 2 responses and Th2-high asthma.

Although epithelial gene expression profiling is a useful research tool, it is important to develop less invasive methods for identifying endotypes, predicting response to therapy with existing agents, and identifying appropriate participants for clinical trials of novel agents. We found that plasma IL-25 correlated with bronchial epithelial IL-25 expression in our study population. Elevated plasma IL-25 levels

normalized after corticosteroid inhalation, which suggests that increases in plasma IL-25 reflect increased IL-25 production in the airways. Like epithelial IL-25 mRNA, plasma IL-25 was elevated in patients with eosinophilic inflammation. More importantly, elevated plasma IL-25 identified a group of subjects with physiologic responses to ICS (increased FEV_1 and decreased hyperresponsiveness), whereas we found no detectable improvement in these measures in subjects with lower plasma IL-25 levels. Plasma IL-25 measurement therefore represents a novel tool for classifying subjects with asthma. Recent studies have demonstrated the value of serum measurements of periostin, a protein that is induced by IL-13 stimulation of epithelial cells, in identifying Th2-high asthma and predicting responses to ICS (29) and to anti–IL-13 antibody (36, 37). Our work suggests the possibility that measurements of IL-25 alone or in combination with measurements of other markers, such as periostin, could help to more precisely define asthma endotypes and identify appropriate cohorts for testing treatments specifically aimed at IL-25/Th2-driven asthma.

Our study has several limitations. First, although plasma IL-25 levels were measured before and after ICS treatment, bronchoscopy and bronchial epithelial brushing was performed only once before ICS treatment. Therefore, the direct effect of ICS treatment on bronchial epithelial IL-25 expression is unknown. Second, because plasma IL-25 level was decreased after ICS treatment in our study population, the utility of IL-25 measurements in subjects using inhaled (or systemic) corticosteroids is uncertain. Some subjects with severe asthma have persistent Th2 responses despite ICS and do benefit from anti–IL-13 antibody therapy (37), and further studies are required to determine whether IL-25 measurements are useful in severe asthma. Third, all subjects in this study were Chinese and most were allergic young adults. Additional studies are required to determine whether phenotyping of asthma based on IL-25 expression is useful in other populations of subjects with asthma. Fourth, our study was an 8-week observational study; longer clinical trials are required to determine whether IL-25 status is a useful predictor of the ability of ICS or other treatments to affect clinically important outcomes, such as rate of exacerbations. Fifth, because of the lack of drugs that specifically target IL-25, we were not able to directly determine whether increased IL-25 expression is required for development or persistence of Th2-high asthma. IL-25 antagonists are effective in mouse models of asthma (21) and our results suggest that plasma IL-25 may be useful for identifying a subset of subjects most likely to benefit from IL-25 antagonists if these become available for testing in humans. Finally, our study was not designed to identify the factors that lead to increased IL-25 expression in some subjects with asthma. IL-25 was induced following allergen challenge of subjects with allergic asthma (25) and mouse studies suggest that this effect may be at least partially caused by allergen proteases (38). Little is known about how epithelial IL-25 expression is affected by other environmental exposures, such as particulates and respiratory viruses. Our results provide additional motivation for further investigation of environmental and genetic factors that may increase IL-25 expression and drive Th2-high asthma. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank the patients who volunteered for this study; Zhenxiang Zhang, Shengdao Xiong, and Hong Huang for referring subjects with asthma for the study; Xiaoling Rao and Zhengyun Wang for bronchoscopy support; Shixin Chen, Wang Ni, and Kun Zhang for spirometry measurement; and Prescott G. Woodruff (University of California San Francisco) for advice in calculation of the three-gene-mean.

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