Gene Expression Patterns of Th2 Inflammation and Intercellular Communication in Asthmatic Airways

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Asthma has been described as an allergic disorder, with airway pathophysiology resulting from chronic Th2-driven eosinophilic inflammation (1). However, this description does not capture the complexity of clinical asthma, which exhibits heterogeneous pathophysiology and pharmacologic responsiveness related to the type of cellular inflammation in the airway (2–4). In particular, the nature and intensity of granulocytic infiltrates (e.g., eosinophilic, neutrophilic, mixed, paucigranulocytic) may define distinct subtypes of asthma (5, 6). Molecular phenotyping of diseased tissues, using technologies such as gene expression microarrays, has the potential to provide insights into the phenotypic heterogeneity of disease and the identification of associated biomarkers (7) or strategies to select patients with an increased potential to respond to molecularly targeted therapies.

New investigational therapeutics for asthma targeting inflammatory cytokines are emerging examples of the potential advantage of patient selection. IL-5 is associated with the expansion, priming, recruitment, and prolonged tissue survival of eosinophils (8), and it has therefore been the subject of study as a potential drug target. Although early studies of IL-5 antagonism in asthmatics failed to show signs of efficacy (9–11), recent clinical studies in prespecified populations of eosinophilic asthmatics have demonstrated benefit (12, 13). Similarly, recent clinical trials of Abs targeting proinflammatory cytokines thought to contribute to asthma pathogenesis, such as TNF-α or IL-4 and IL-13 (1, 14, 15), failed to meet their primary endpoints in all comers, but in post hoc analyses there was evidence suggesting that subgroups of asthmatics experienced enhanced benefit (16, 17). However, the efficacy of molecularly targeted therapies in a clinical setting depends on both appropriate patient selection and appropriate outcome selection. These studies highlight the importance of understanding the underlying basis of heterogeneity in disease and the relationships between targeted pathways and in vivo pathophysiology for developing strategies to identify patient populations with maximal potential benefit from molecularly targeted therapies.

We have previously reported molecular signatures associated with clinical subphenotypes of asthma (18). We dichotomized a mild-to-moderate asthmatic population into “Th2-high” and
“Th2-low” subphenotypes on the basis of a signature of three IL-13 inducible genes in bronchial epithelial brushings. These subphenotypes exhibited distinct pathology and corticosteroid responsiveness. Molecular phenotyping of airway epithelium is therefore informative and relevant, as it describes a molecular basis for asthma pathophysiology (19). However, the pathophysiology of asthma is not limited to the epithelium. Endobronchial biopsies permit direct analysis of epithelial and subepithelial cellular and molecular mediators of asthma, including structural cells of the airway and inflammatory infiltrates. In the current study, our objective was to perform gene expression microarrays analyses of endobronchial biopsies matched to previously characterized bronchial epithelial brushings, directly comparing Th2 subphenotypes. It is the premise of these analyses that complementary molecular phenotyping could elucidate a more granular description of the pathophysiological mediators of Th2 inflammation in asthma.

**Materials and Methods**

**Subjects**

Bronchial biopsy RNA from 27 mild-to-moderate nonsmoking asthma patients and healthy nonsmoking subjects was obtained from the University of California, San Francisco, Airway Tissue Bank, a specimen biorepository approved by the University of California, San Francisco, Committee on Human Research and managed by two of the authors (J.V.F. and P.G.W.). Endobronchial biopsies had been collected from a subset of patients in whom we have previously described gene expression profiles in bronchial epithelium (as described in detail elsewhere; see Refs. 18, 20). Three to six endobronchial biopsies were collected from the ca- raina of second- to fourth-order bronchi, and the RNA was pooled after extraction using methods we have previously described. The clinical characteristics of the 13 healthy controls and 27 asthmatic subjects described in this study are shown in Table I. Informed consent was obtained from all human subjects after the nature and possible consequences of the study were explained.

**Gene expression analyses**

RNA was isolated from homogenized bronchial biopsies and quantitative real-time PCR (qPCR) for IL-5 and IL-13 was performed as described previously (18). TaqMan gene expression assays (Applied Biosystems, Foster City, CA) were purchased and conducted per the manufacturer’s instructions for CST1 (i.d. Hs00606961_m1), MUC5B (i.d. Hs00861595_m1), CLCA1 (i.d. Hs00554490_m1), and CCL26 (i.d. Hs00171146_m1). RNA was amplified (MessageAmp II; Ambion, Austin, TX) for Agilent (Santa Clara, CA) two-color Whole Human Genome 4 × 44K gene expression microarray analysis. Universal Human Reference RNA (Stratagene, La Jolla, CA) was used for the reference channel. Probe intensities were transformed as log2 ratios of test and reference channels calculated for CCGf, using bronchial epithelial samples. CCL26 was the most highly differentially expressed gene between Th2-high asthma and Th2-low asthma and control, constituting 93 probes (q < 0.05) corresponding to 79 uniquely annotated genes (Supplemental Table I).

We verified a subset of the DE probe list by qPCR (Supplemental Table III). Among the verified genes were several that have been previously associated with asthma and/or Th2 inflammation: chloride channel, calcium-activated, family member 1 (CLC1A1); mucin 5B, oligomeric mucus/gel-forming (MUC5B); and chemokine (C-C motif) ligand 26 (CCL26). CLCA1 is upregulated in airway epithelial cells by IL-13 stimulation in vitro and is expressed at elevated levels in vivo in asthmatic bronchial epithelium (20), which may have an indirect role in mediating calcium-activated chloride currents and mucus secretion (23). MUC5B is a member of a family of mucin glycoproteins produced by goblet cells in the lung that contribute to the viscoelastic and adhesive properties of airway mucus, and it has been shown to be downregulated in the airways of asthmatics, with concomitant upregulation of MUC5AC and MUC2 (18, 24).

CCL26 was the most highly differentially expressed gene between Th2-high asthma and Th2-low asthma and control by microarray (fold change = 4.06, q = 1.3 × 10^-5) and confirmed by qPCR (Supplemental Table III). CCL26, also known as eotaxin-3, is a chemokine that binds to CCR3 and acts as a potent eosinophil chemoattractant. Stimulation by the Th2 cytokines IL-4 and IL-13 in vitro systems strongly upregulates CCL26 expression in human bronchial epithelial cells (25) and PBMCs (26). CCL26 protein has been reported to be elevated in asthmatic bronchial mucosa (25). Furthermore, in a study of eosinophilic esophagitis, a Th2-associated disease of the esophageal mucosa, CCL26 was observed to be the mostly highly induced gene, whose expression strongly correlated with tissue eosinophilia and mastocytosis (27). We assessed the correlation between biopsy CCL26 gene expression and cytokine expression (quantified by qPCR) and observed strong positive correlations with the Th2 cytokines IL-5

**Results**

Differentially expressed bronchial biopsy genes are highly intercorrelated and relate directly to clinical measures of allergy and inflammation

To identify patterns of gene expression in bronchial mucosa corresponding to Th2 inflammation, we performed differential gene expression analysis in bronchial biopsies from 27 asthmatics and 13 healthy control subjects using discrete categorizations of bronchial epithelial Th2 signature status as prespecified stratification criteria. On the basis of a three-gene bronchial epithelial expression pattern, we have previously categorized these subjects into two clusters: one comprising Th2-high asthma and one comprising Th2-low asthma and healthy controls (18). A two-sample t test (see Materials and Methods) was employed to generate a DE probe list for Th2-high asthma versus Th2-low asthma and control, constituting 93 probes (q < 0.05) corresponding to 79 uniquely annotated genes (Supplemental Table I).

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(rho = 0.48, p = 0.002) and IL-13 (rho = 0.79, p < 0.0001), but not with IL-4 (not shown), and a strong negative correlation with the Th1 cytokine IL-12A (rho = −0.53, p = 0.0006) (Fig. 1A).

CCL26, IL-5, and IL-13 are widely held to be key effector molecules in the manifestation of Th2 inflammation in asthma (14, 15). Comparing quantitative local and systemic phenotypic assessments indicative of allergic inflammation (Fig. 1B), we found positive correlations between CCL26 gene expression and serum IgE (rho = 0.64, p < 0.0001), peripheral blood eosinophil count (rho = 0.53, p = 0.0005), and a trend for association with eosinophil percentage in bronchoalveolar lavage (BAL) fluid (rho = 0.30, p = 0.064). Additionally, we noted several other genes among the DE probes known to be associated with Th2 inflammation: IgE; NO synthase 2A (NOS2A); histamine receptor H1; GPR44, also known as chemoattractant receptor-homologous molecule expressed on Th2 cells; and arachidonate 15-lipoxygenase (ALOX15) (18, 28–33). Taken together, the differential expression of these genes is consistent with the phenotypic descriptions of Th2-high and Th2-low asthma.

We observed a high degree of intercorrelation among the probes in the DE probe list described in Supplemental Table I. Two-way similarity clustering (Spearman correlation) of the 93 probes resulted in two major clusters of positively correlated probes (Fig. 1C). The clusters were anticorrelated with one another. Considering the high degree of intercorrelation and the direct relationship between CCL26 (probe i.d. A_24_P12573), qPCR assessments of IL-5, IL-13, and IL-12A, and local and systemic clinical measures of allergy and inflammation, it was not surprising to note a high prevalence of significant (q < 0.05) associations of these measures with individual expression of each DE probe. With the exception of eosinophil percentage in the BAL, each of these measures was significantly correlated (q < 0.05, Spearman correlation) with each individual DE probe, whereas BAL eosinophil percentage was correlated with 88 of 93 (95%) of the probes (Supplemental Table I).

Taken together, these data suggest that airway gene expression of Th2-associated molecules, such as CCL26, IL-5, IL-13, and other genes identified by differential gene expression analysis, have a direct and continuous relationship with local and systemic markers of Th2 inflammation in asthmatics. Due to the high degree of intercorrelation among the DE genes and direct correlation with clinical measures of Th2 inflammation, we hypothesized that a variable continuum of gene expression underlies discrete Th2-low and Th2-high phenotypes determined by bronchial epithelial gene expression (18) and that these DE genes described a Th2 inflammation signature gene set. To test this hypothesis, we: 1) developed a Th2 sig for the 93 DE probes and evaluated it against molecular and clinical markers of Th2 inflammation, and 2) assessed this summary metric against the biological space described by CCGF to more comprehensively describe the molecular processes associated with Th2 inflammation in asthmatic airways.

The biopsy Th2 sig describes a molecular and clinical continuum of Th2 inflammation

We summarized the expression of the 93 DE probes into a single continuous classifying metric by SPCA (34, 35). Scores from PC1, representing 49% of the variance of the highly intercorrelated Th2 signature gene set, are used as scalar values for the Th2 sig. As expected, Th2-high asthma is distinguished from control, exhibiting minimal overlap with Th2-low asthma along the PC1 axis (Supplemental Fig. 2). Organized by PCA factors, an intensity heat map of normalized DE probe intensity depicts an intuitive subject hierarchy on the basis of a coordinated continuum of Th2 signature gene expression (Fig. 2). Specifically, upregulated Th2-high genes, such as CCL26, CLCA1, and IgE, are coordinately expressed from low to high levels from Th2-low to Th2-high phenotypes determined by bronchial epithelial expression (18) and that these DE genes described a Th2 inflammation signature gene set. To test this hypothesis, we: 1) developed a Th2 sig for the 93 DE probes and evaluated it against molecular and clinical markers of Th2 inflammation, and 2) assessed this summary metric against the biological space described by CCGF to more comprehensively describe the molecular processes associated with Th2 inflammation in asthmatic airways.

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FIGURE 1. Relationship among differentially expressed bronchial biopsy genes and clinical measures of allergy and inflammation. CCL26 was the most highly differentially expressed gene between Th2-high asthma and Th2-low asthma and control (fold change = 4.06, q = 1.3 × 10−5) and is directly associated with Th2 inflammation. A. CCL26 (probe i.d.: A_24_P12573) positively correlates (Spearman rank order) with qPCR assessments of IL-5 (rho = 0.48, p = 0.002) and IL-13 (rho = 0.79, p < 0.0001) and negatively with IL-12A (rho = −0.53, p = 0.0006). B. CCL26 (probe id: A_24_P12573) correlates (Spearman rank order) with serum IgE (rho = 0.64, p < 0.0001) and peripheral blood eosinophil count (rho = 0.53, p = 0.0005) and and weakly associates with BAL fluid eosinophil percentage (rho = 0.30, p = 0.064). C. Similarity clustering analysis of DE probes (probe versus probe) illustrates a high degree of expression intercorrelation (Spearman rank order) among the DE probe list (Supplementary Table I). DE probes in Th2-high asthma are indicated by adjacent red (upregulated) and blue (downregulated) rectangles.
characteristic analysis (Fig. 3). Taken together, these analyses suggest that Th2 inflammation in underlying bronchial mucosa above a threshold level is necessary to trigger the dramatic phenotypic differences we observed between groups for bronchial epithelial gene expression, airway remodeling, and corticosteroid responsiveness (Table I).

Consistent with the observation that individual genes in the Th2 SIG have a direct relationship with clinical measures of allergy and inflammation, Th2 SIG, which summarizes the collective expression of 93 probes encoding 79 uniquely annotated genes, significantly correlates with qPCR assessments of IL-5 (rho = 0.45, \( p = 0.0038 \)), IL-13 (rho = 0.74, \( p < 0.0001 \)), and IL-12A (rho = 0.71, \( p < 0.0001 \)); serum IgE (rho = 0.62, \( p < 0.0001 \)); blood eosinophil count (rho = 0.53, \( p = 0.0005 \)); and BAL eosinophil percentage (rho = 0.39, \( p = 0.017 \)) (Fig. 4).

CCGF gene correlations with the biopsy Th2 SIG

Asthma has been described as an allergic disorder, and the molecular effectors of Th2 inflammation have largely been described in terms of the biological space of CCGF due to their direct relevance as effector molecules in inflammation, cell migration, and tissue remodeling in addition to their relative tractability as therapeutic targets (1). We performed a focused correlation analysis of a manually curated gene set of known CCGF versus Th2 SIG (Fig. 5, Supplemental Table II) within asthmatics to further elucidate the association between molecular and clinical components of Th2 inflammation in asthma and describe the Th2 inflammatory phe-

FIGURE 3. Logistic regression of bronchial epithelial Th2 phenotype by biopsy Th2 SIG. Ordinal logistic regression was performed on bronchial epithelial Th2 phenotype, as defined previously (18), by biopsy Th2 SIG. A high degree of association was observed \( (p < 0.0001) \) between the two metrics. A, Regression and (B) receiver operating characteristic plots from regression model.
notype more broadly within the biological space of intercellular mediators with generally well-characterized functions. Limiting our analysis to CCGf also serves as a useful analytical strategy to minimize multiple test correction penalties.

Among 212 genes encoding uniquely annotated CCGf (268 probes), 25 (27 probes) were positively or negatively correlated (Spearman correlation) with the Th2 sig below a $q$ value threshold of 0.05, and 45 (49 probes) were correlated with a $q$ value below a threshold of 0.10 (Fig. 5). Unsurprisingly, CCL26 and IL-13 were positively correlated with the Th2 sig, as was the CCR3-binding eosinophil attracting chemokine CCL13 (36). CCGf whose expression levels were strongly negatively associated with the Th2 sig included the Th1 cytokine IL-12A and the Th1 chemokine CXCL11 (37–40). Taken together, these findings suggest that Th2 inflammation may occur in the context of suppressed Th1 inflammation in asthma and further support the concept that the Th2 sig is a quantitative metric of Th2 burden in the airway. In addition to positive correlations between the Th2 sig and CCR3-binding eosinophil-attracting chemokines CCL13 and CCL26, we observe strong negative correlations between the Th2 sig and the CXCR1/2-binding neutrophil-attracting chemokine CXCL6, as well as the neutrophil hematopoietic factor CSF3 (G-CSF), which may underlie differences in airway granulocyte infiltration described for asthma subphenotypes (5, 6).

We observed positive correlations between diverse groups of inflammatory factors and the Th2 sig. TNF-α is produced along with Th2 cytokines from “inflammatory Th2” cells stimulated by OX40L-expressing dendritic cells (41), and we have previously shown that BAL macrophages from Th2 high asthmatics express elevated levels of TNF-α (18). Positive correlations between the Th2 sig and TNFRSF4 (OX40) and TNFRSF9 (4-1BBL) suggest infiltration of activated helper T cells, which are likely sources of

### Table I. Clinical and demographic characteristics of the study population

<table>
<thead>
<tr>
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<th>Healthy Control</th>
<th>Asthma</th>
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<tbody>
<tr>
<td>Sample size</td>
<td>13</td>
<td>27</td>
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<tr>
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<td>33 (20–55)</td>
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<td>Gender, M/F</td>
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<tr>
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<td>3</td>
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<tr>
<td>FEV1, % predicted</td>
<td>102 (92–136)</td>
<td>87.7 (65–107)</td>
</tr>
<tr>
<td>Methacholine PC20</td>
<td>64 (21.5–64)</td>
<td>1.4 (0.05–7.27)</td>
</tr>
<tr>
<td>IgE (IU/ml)</td>
<td>23 (3–177)</td>
<td>221 (19–2627)</td>
</tr>
<tr>
<td>Blood eosinophils ($\times 10^9/l$)</td>
<td>0.085 (0.03–0.28)</td>
<td>0.27 (0.07–0.94)</td>
</tr>
<tr>
<td>BAL eosinophils (%)</td>
<td>0.2 (0–0.6)</td>
<td>0.5 (0–7.4)</td>
</tr>
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Values are presented as median (range).

FEV1, forced expiratory volume in 1 s; PC20, provocative concentration required to cause a 20% decline in forced expiratory volume in 1 s.

**FIGURE 5.** Correlation of asthma Th2 sig with CCGf. Correlation analysis (Spearman rank order) was performed among asthmatics ($n = 27$) with a manually curated list of CCGf. Correlation significance is plotted (all) and annotated ($q < 0.1$) by strip chart. Positive correlations are annotated in red to the right of the midline. Negative correlations are annotated in blue to the left of the midline.

**FIGURE 4.** Characteristics of the Th2 sig. The Th2 sig relates directly to molecular and clinical measures of allergy, inflammation, and lung function. A, Correlation (Spearman) of the Th2 sig with qPCR assessments of IL-5 (rho = 0.45, $p = 0.0038$), IL-13 (rho = 0.74, $p < 0.0001$), and IL-12A (rho = -0.71, $p < 0.0001$). B, Correlation (Spearman) of the Th2 sig with serum IgE (rho = 0.62, $p < 0.0001$), blood eosinophil count (rho = 0.53, $p = 0.0005$), and BAL eosinophil percentage (rho = 0.39, $p = 0.017$).
many of the inflammatory cytokines observed (42). KITLG, also known as stem cell factor, is a mast cell growth factor and chemoattractant (43). LIF is a member of the IL-6 family of cytokines binding to the shared gp130 subunit of the IL-6R complex. Its expression is positively correlated with the Th2 sig and has been linked to airway remodeling in preclinical models (44). Taken together, these findings describe a complex interplay between mast cells, T cells, APCs, and airway stroma contributing to the Th2 inflammatory phenotype.

In addition to a large number of inflammatory cytokines and chemokines, several additional families of growth factors associated with epithelial–mesenchymal communication and tissue remodeling were correlated with the Th2 sig, including Wnt, TGF-β, and platelet-derived growth factor (PDGF) family members. Multiple Wnt genes were positively (Wnt3A, Wnt5A, Wnt6, and Wnt10A) or negatively (Wnt5B) correlated with the Th2 sig. Additionally, Fzd5, a Wnt receptor, is on the initial list of DE genes and is upregulated in Th2-high asthma (Supplementary Table I). WntS have not previously been reported as being differentially expressed in human asthmatic airways, although Wnt5A can be induced by IL-13 in vitro in PBMCs (26) and by IL-6 family cytokines (45). Similarly intriguing patterns emerge with TGF-β family members, including TGF-β1, BMP7, inhibins A and C, and GDFs 1 and 3, which are positively correlated with Th2 sig, whereas INHB is negatively correlated with the Th2 sig; as well as with PDGF family members, including PDGFs A and B, HGF, VEGFC, and the aforementioned KITLG, which are positively correlated with the Th2 sig, whereas TGFα is negatively correlated with the Th2 sig. Although many of these factors have been individually associated with allergic inflammation in vitro, in preclinical animal models, and, to a lesser extent, in vivo in human asthma, we present in this study a comprehensive overview of the relationships between key mediators of inflammation, cellular migration, and tissue remodeling as they relate to quantitative measures of allergic airway inflammation.

**Discussion**

Through gene expression profiling of asthmatic bronchial biopsies, directly comparing Th2 subphenotypes (18), we have found that highly differentially expressed asthma genes are part of a coordinated pattern of gene expression whose biological function and magnitude correspond to local and systemic measures of allergy and Th2 inflammation. We have summarized the expression of the Th2 inflammation gene set into a Th2 sig. The direct relationship that we observed between the Th2 sig and clinical manifestations of Th2 inflammation is consistent with a paradigm in which Th2 inflammation, both in molecular and cellular terms, exhibits a continuum of expression in asthma. Having established that the Th2 sig is a quantitative expression-based depiction of Th2 inflammation, we were able to comprehensively explore relationships between key intercellular mediators of inflammation, cell migration, and tissue remodeling directly in human asthmatic bronchial tissue.

There is a relative paucity of published whole genome expression studies of human asthmatic bronchial tissue, despite the potential insights that such studies may afford (46), thus precluding a comprehensive comparison of our findings with other datasets. Comprehensive expression analyses have been performed and published in experimental mouse models of asthma, and when comparing the report of upregulated pulmonary expression by IL-4, IL-13, and allergen challenges from one such study (47), we observe minimal overlap or consistency of direction of regulation for implicated asthma genes (Supplemental Fig. 3A). A small whole genome expression study of bronchial biopsies involving four mild asthmatics identified 79 genes differentially expressed in asthma as compared with normal controls (48). Although only two genes (NOS2A and ALOX15) in that study are in common with our analyses above a nominal significance threshold of $q < 0.05$, we find a greater consistency in the direction of dysregulation than in comparison with mouse models (Supplemental Fig. 3B). The small number of subjects in that study and the degree of heterogeneity we have demonstrated in our larger cohort may contribute to the lack of overlap between studies. Interestingly, when comparing the genes reported to be differentially expressed in eosinophilic esophagitis, a Th2-associated disease of the esophageal mucosa (27), we observe a substantial intersection of genes with our DE list (Supplemental Fig. 3C), which suggests that common molecular and pathophysiological mechanisms underlie the mucosal inflammation observed in eosinophilic esophagitis and Th2-high asthma.

**Biopsy Th2 sig**

We addressed intrinsic heterogeneity in our asthma biopsy gene expression data set by using bronchial epithelial three-gene Th2 signature status (18) as a t test factor. We used this approach as a means to identify bronchial biopsy genes that are differentially expressed specifically in Th2-high asthmatics. As we had evidence that the differentially expressed genes represented a continuum of expression across the data set, we used SPCA (34, 35) to derive a biopsy Th2 signature as a continuous metric. An alternative classification technique to hierarchical clustering of microarray data, SPCA has been applied in situations when a continuous metric is desired (49). Through linear combinations of original variables, PCA projects the variance of correlated variables into a reduced number of dimensions without loss of information, that is, orthogonal principal components.

The genes that comprise the Th2 signature are highly coordinately regulated. The metric likely includes the effects of varying tissue cytology, where the unique transcriptional profiles of leukocytes, epithelial cells, and stromal cells of differing lineage, states of differentiation, and activation (50–52) contribute to the overall biopsy molecular profile. Therefore, individual genes within the Th2 sig may variably contribute to, or result from, components of asthma pathophysiology, but their coordinate regulation in asthmatic airways is consistent with the dramatic pathophysiological effects observed in asthma. In summary, we interpret the Th2 sig as a quantitative proxy for the net pathological state associated with airway Th2 inflammation. Note that this approach explicitly seeks to identify gene expression changes linked to Th2 inflammation. The possibility remains that orthogonal patterns of differential gene expression may underlie other pathophysiological features or subphenotypes of asthma.

Distinct subphenotypes of asthmatics have been described on the basis of the presence or absence of eosinophilic airway inflammation (6, 53, 54). A concept of discrete asthma subphenotypes driven by allergic inflammation or other factors may suggest disparate disease processes with a common final pathology of airway hyperreactivity and reversible obstruction. In a previous study of this cohort of asthmatics, we described distinct subphenotypes of asthma based on a limited three-gene signature in the airway epithelium. Dichotomizing asthmatics according to this signature into Th2-high and Th2-low subphenotypes is pathophysiologically and clinically relevant, as there are clear distinctions in terms of airway remodeling and mucus composition. Importantly, these subphenotypes have dramatic differences in their responsiveness to inhaled corticosteroid treatment, with improvements in airway function observed only in the Th2-high subset. However, allergic inflammation as measured by aeroallergen
sensitivity and serum IgE was still evident, if at lower levels, in the Th2-low subset (18). Taken together with our observations in bronchial mucosal biopsies in the current study, these findings suggest that a threshold level of underlying Th2 inflammation in the bronchial mucosa may be required to trigger the dichotomous epithelial gene expression and pathological and clinical phenotypes we have described. Although thresholds are essential for assigning subjects to nominal categories to assess clinical outcomes, our explicit objective in the current study was to comprehensively characterize gene expression related to Th2 inflammation in asthmatic bronchial tissue, and hence a continuous, quantitative measure such as the Th2 sig serves as a useful tool to accomplish this end.

**Intercellular communication and asthma pathophysiology**

We selectively examined the Th2 sig with respect to the expression of CCGf because this biological space comprises a broad but manageable number of well-characterized factors that mediate intercellular communication. Although many of the CCGf we identified as being coexpressed with the Th2 sig have been described as associated with Th2 airway inflammation via gene expression analyses using in vitro cell culture or preclinical animal models, this is to our knowledge the first attempt to characterize them globally in human asthmatic airway tissue. We found a consistent pattern of upregulated Th2 and eosinophil-attracting cytokines and chemokines (e.g., IL-13, CCL13, CCL26) with concomitant downregulation of Th1 and neutrophil-attracting cytokines and chemokines (e.g., IL-12A, CSF3, CXCL6, CXCL11). The finding that TNF-α and OX40 (TNFRSF4) expression are significantly coexpressed with Th2 inflammation in human asthma supports the notion that pathologic Th2 inflammation may stem from a nonclassical inflammatory Th2 phenotype, which may be driven by OX40L–OX40 interactions (41). The respective positive and negative correlations between known Th2 and Th1 mediators and the Th2 sig illustrates a broad spectrum of mediators that actively play a role in bronchial mucosal inflammation and add confidence that other coexpressed genes are indeed relevant to Th2 inflammation in human asthma.

Airway remodeling, including reticular basement membrane thickening, smooth muscle hyperplasia, mucous metaplasia, and fibrosis, is thought to be an incompletely reversible long-term consequence of allergic airway inflammation, which may contribute to the progressive decline in pulmonary function observed over the course of many years in some asthmatics (55). We have previously shown that the epithelial Th2 signature is positively correlated with reticular basement membrane thickness, an index of bronchial fibrosis (18). Our findings of distinctive and coordinated expression patterns of Wnt, TGF, and PDGF family members coexpressed positively or negatively with the Th2 sig suggest an active process of tissue remodeling associated with Th2 inflammation. In particular, we noted that TGF-β1 expression is positively correlated with the Th2 sig, consistent with other reports linking TGF-β expression with eosinophilic airway inflammation (56). Equally striking were the correlations between its cognate receptor Fzd5 (57) and Th2-high asthma (Fig. 2, Supplementary Table I). Wnt5A is highly expressed in fibroblasts isolated from subjects with idiopathic pulmonary fibrosis (58), which is intriguing in light of the fact that subepithelial fibrosis is significantly greater in Th2-high asthma as compared with Th2 low asthma (18). Given their established roles in tissue development, cellular differentiation, and patterning, it is tempting to speculate that Wnts may be involved in airway remodeling characteristic of allergic asthma. A recent study of therapeutic IL-5 blockade in severe eosinophilic asthmatics found a small but significant decrease in bronchial wall thickness over a 1-y period (12). With a large and growing number of novel therapeutic candidates targeting Th2 inflammation currently in development (15, 59, 60), it will be important to assess the ability of these molecules to modulate the expression of factors that regulate tissue remodeling and link those effects to pathophysiological and clinical outcomes.

We conducted this study in a cohort of mild-to-moderate asthmatics who were not currently taking steroids. An advantage of this approach is that, since our objective was to characterize patterns of gene expression associated with Th2 inflammation in human asthma, the potential confounding effects of steroids on gene expression were minimized. However, the most significant unmet medical need in asthma and the target population for emerging therapies directed against Th2 inflammation is more severe asthmatics whose disease is poorly controlled despite steroid treatment (15, 59, 60). In moderate asthmatics who require inhaled corticosteroids to control their disease, it appears that Th2 inflammation as assessed by airway IL-13 levels is largely suppressed, whereas in severe asthmatics uncontrolled despite steroid treatment, a subset with elevated airway IL-13 levels emerges (61). We have found that while Th2-high mild-to-moderate asthmatics respond well to inhaled corticosteroids, Th2-low subjects do not (18). Thus, it appears likely that severe asthmatics refractory to the effects of inhaled corticosteroids may have acquired resistance to the action of steroids, pathology driven by mechanisms not inherently susceptible to steroids, or a combination of the two. These mechanistic considerations will be important for understanding the clinical consequences of emerging therapies targeting Th2 inflammation in severe asthma. Now that we have laid the groundwork for understanding patterns of Th2 inflammation in asthmatic airways in the absence of steroids, future efforts should be directed at characterizing this pathway in the context of therapeutic interventions, including steroid treatment and new targeted therapeutics directed against components of Th2 inflammatory pathways.

In clinical studies, asthma therapies that specifically target components of Th2 inflammation, such as anti-IgE (omalizumab) or anti–IL-5 (mepolizumab), confer clinical benefits in the context of allergen challenge or exacerbation outcomes but not on airway obstruction outcomes, such as forced expiratory volume in 1 s (4, 9–13, 15, 29, 62–64). Our findings suggest that a predominant pattern of differential gene expression in asthma is related to Th2-driven airway inflammation; however, this pathway is linked to a large number of other factors associated with aspects of airway pathophysiology. Although Th2 inflammation is hypothesized to be a driver of disease in allergic asthma, it is as yet unclear whether Th2 inflammation is a cause or a consequence of the extended network of inflammatory and regulatory factors described in this study. Thus, it will be important to assess the clinical benefit of therapies targeting Th2 pathways on outcomes that are relevant to Th2 inflammation. This raises several important implications for future studies: 1) by defining components of asthma related to Th2 inflammation, we may now seek to identify components of asthma not related to Th2 inflammation that may underlie airway obstruction and hyperreactivity; 2) by relating distinct patterns of airway gene expression to specific domains of asthma pathophysiology, we may better interrogate the value of therapeutic interventions on clinical outcomes that are most relevant to the pathways being targeted; 3) by quantifying the degree to which Th2 inflammation is active in individual asthmatics, we may better identify patients most likely to benefit from therapeutics targeting Th2 inflammation; and 4) by developing a comprehensive, quantitative metric of Th2 airway inflammation, we are now equipped to interrogate the effectiveness of specific
molecular interactions on the large-scale pattern of gene expression associated with Th2 inflammation in asthma.

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Disclosures


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