Identification of airway mucosal type 2 inflammation by using clinical biomarkers in asthmatic patients

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Background: The Airways Disease Endotyping for Personalized Therapeutics (ADEPT) study profiled patients with mild, moderate, and severe asthma and nonatopic healthy control subjects.
Objective: We explored this data set to define type 2 inflammation based on airway mucosal IL-13–driven gene expression and how this related to clinically accessible biomarkers.
Methods: IL-13–driven gene expression was evaluated in several human cell lines. We then defined type 2 status in 25 healthy subjects, 28 patients with mild asthma, 29 patients with moderate asthma, and 26 patients with severe asthma based on airway mucosal expression of (1) CCL26 (the most differentially expressed gene), (2) periostin, or (3) a multigene IL-13 *in vitro* signature (IVS). Clinically accessible biomarkers included fraction of exhaled nitric oxide (FENO) values, blood eosinophil (bEOS) counts, serum CCL26 expression, and serum CCL17 expression.
Results: Expression of airway mucosal CCL26, periostin, and IL-13–IVS all facilitated segregation of subjects into type 2–high and type 2–low asthmatic groups, but in the ADEPT study population CCL26 expression was optimal. All subjects with high airway mucosal CCL26 expression and moderate-to-severe asthma had FENO values (≥35 ppb) and/or high bEOS counts (≥300 cells/mm^3) compared with a minority (36%) of subjects with low airway mucosal CCL26 expression. A combination of FENO values, bEOS counts, and serum CCL17 and CCL26 expression had 100% positive predictive value and 87% negative predictive value for airway mucosal CCL26–high status. Clinical variables did not differ between subjects with type 2–high and type 2–low status. Eosinophilic inflammation was associated with but not limited to airway mucosal type 2 gene expression.
Conclusion: A panel of clinical biomarkers accurately classified type 2 status based on airway mucosal CCL26, periostin, or IL-13–IVS gene expression. Use of FENO values, bEOS counts, and serum marker levels (eg, CCL26 and CCL17) in combination might allow patient selection for novel type 2 therapeutics. (J Allergy Clin Immunol 2017;140:710-9.)

Key words: *Asthma, type 2 inflammation, phenotypes, airway mucosal gene expression, biomarkers*

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Asthma is a heterogeneous disease characterized by chronic airway inflammation.[1] Although there are many proposed asthma phenotypes, the underlying phenotypes are poorly understood and hard to identify, making tailored asthma treatment an even greater challenge.

Type 2 inflammation in asthmatic patients (also termed T_H2 inflammation) refers to inflammation driven by type 2 cytokines, such as IL-4, IL-5, and IL-13, secreted by $CD4^+$ T_H2 cells, as recently reviewed.[2] Type 2 innate lymphoid cells have also been proposed to secrete the same cytokines.[3] Single and multiple gene signatures in airway brushings and sputum inflammatory cells have recently been used to define type 2–high or type 2–low status.[4-6]

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Supported by Janssen R&D, Spring House, Pa.

Disclosure of potential conflict of interest: P. E. Silkoff and M. J. Loza are employed by Janssen R&D and have stock options from J&J. D. Singh's institution has received a grant from Johnson and Johnson, and he has received consultancy fees from Almirall, AstraZenca, Boehringer Ingelheim, Chiesi, Glenmark, Merck, NAPP, Novartis, Pfizer, Takeda, Teva, Therevance, and Verona and payment for lectures from AstraZeneca, Boehringer Ingelheim, Chiesi, GlaxoSmithKline, Glenmark, Merck, NAPP, Novartis, Pfizer, Takeda, Teva, Therevance, Verona, SkyePharma and Genentech. J. M. FitzGerald's institution has received a grant from UBC. P.-O. Girodet has received personal fees from Novartis, Boehringer Ingelheim and AstraZeneca and has received nonfinancial support from Novartis, Chiesi, Takeda and Boehringer Ingelheim. P. Berger reports other support from Jansen and Jansen during the conduct of the study; personal fees and nonfinancial support from Novartis; grants, personal fees, and nonfinancial support from GlaxoSmithKline; grants from Pierre Fabre; personal fees and nonfinancial support from Takeda; personal fees and nonfinancial support from Chiesi; personal fees, nonfinancial support, and other support from Boehringer Ingelheim; and personal fees and nonfinancial support from AstraZeneca outside the submitted work. G. Chupp's institution has received money from Yale University, and he has received consultancy fees from GlaxoSmithKline, AstraZeneca, Genentech, and Teva and payment for lectures from Circassia, AstraZeneca, and Boehringer Ingelheim. V. S. Susulic, E. S. Barnathan, and F. Baribaud are all employed by Janssen R&D. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication February 25, 2016; revised November 6, 2016; accepted for publication November 21, 2016.

Available online January 13, 2017.

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0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2016.11.038

Abbreviations used
ADEPT: Airways Disease Endotyping for Personalized Therapeutics
bEOS: Blood eosinophil
CLCA1: Calcium-activated chloride channel regulator 1
FDR: False discovery rate
FENO: Fraction of exhaled nitric oxide
ICS: Inhaled corticosteroid
IVS: *In vitro* signature
NPV: Negative predictive values
PPV: Positive predictive value
sCCL17: Serum CCL17
sCCL26: Serum CCL26
SERPINB2: Serpin peptidase inhibitor, clade B, member 2 (also known as plasminogen activator inhibitor 2)
sIgE: Serum IgE
spEOS: Sputum eosinophil

The most effective therapies for asthma are anti-inflammatory, including inhaled corticosteroids (ICSs) and mAbs, but these do not work in all patients. For example, the response to ICSs is associated with a high fraction of exhaled nitric oxide (FENO) value,[7] high sputum eosinophil (spEOS) count,[8] or 3-gene type 2–high signature in epithelial brushings.[5] Similarly, for the mAbs omalizumab (anti-IgE),[9] dupilumab (anti–IL-4 receptor),[10] mepolizumab and reslizumab (anti–IL-5),[11,12] or lebrikizumab (anti–IL-13),[13] optimal efficacy is seen in patients with severe asthma with type 2 inflammation identified based on biomarkers, including blood eosinophil (bEOS) counts, spEOS counts, FENO values, or serum periostin expression.[9-13]

Notably, despite these patient selection strategies, severe asthma is often incompletely responsive to these and other currently available therapies.[14,15] This might be due to difficulty in defining phenotypes, including the type 2 phenotype, leading to selection of inappropriate therapy or additional mechanisms alone or in addition to type 2 inflammation, which are driving pathology. For example, in a recent report for lebrikizumab, even subjects with high periostin levels had a 95% confidence limit range for improvement in FEV_1 of between 1.0% and 15.4%,[13] illustrating that single biomarkers cannot capture complex biological networks.

The previously reported Airways Disease Endotyping for Personalized Therapeutics (ADEPT) study[16] allowed us to evaluate airway mucosal gene expression associated with type 2 inflammation, including periostin expression, which has been associated with enhanced responses to the anti–IL-13 mAb lebrikizumab[13]; CCL26, which in our hands was the most highly expressed gene in IL-13–treated human cells lines; and a multigene *in vitro* signature (IVS) from the same cell lines (IL-13–IVS). We also ascertained whether type 2 gene expression profiles for CCL26, IL-13–IVS, and periostin could be classified by using clinically accessible biomarkers, namely FENO values, bEOS counts, and less commonly used serum markers, such as CCL17/TARC (thymus and activation-regulated chemokine) and activation-regulated chemokine and CCL26/eotaxin-3, which are type 2 chemokines reliably measured in serum.

METHODS

Study design and population

The ADEPT study design and population (clinicaltrials.gov registration no. NCT01274507) have been described in detail.[16] In brief, nonatopic healthy subjects, patients with mild asthma (no controller medications and prebronchodilator FEV_1 ≥80% of predicted value), patients with moderate asthma (low-to-moderate ICS dose and prebronchodilator FEV_1 60% to <80% of predicted value), and patients with severe asthma (high-dose ICS and prebronchodilator FEV_1 50% to less than 80% of predicted value) were enrolled. Healthy subjects and a subset of asthmatic patients underwent bronchoscopy. All ADEPT study subjects with good-quality airway mucosal tissue from biopsy specimens were included in the biopsy assessment detailed below. Clinical assessments included spirometry, bronchodilator reversibility, methacholine airway hyperresponsiveness, the Asthma Control Questionnaire,[17] and the Asthma Quality of Life Questionnaire.[18] The study was approved by site-specific institutional review boards, and all subjects signed an informed consent form. Healthy and asthmatic subjects attended screening, baseline, and bronchoscopy visits, and asthmatic patients also attended biomarker visits at 3, 6, and 12 months.

Biomarker assessments

Bioanalysis methods are detailed in the Methods section in this article's Online Repository at www.jacionline.org. Briefly, gene expression in RNAlater-preserved biopsy samples was analyzed by means of microarray (HG-U133+PM platform; Affymetrix, Santa Clara, Calif), and histology of biopsy specimens was performed by Pantomics (Richmond, Calif). Induced sputum was collected, processed by using the plug selection method,[19] and analyzed for differential cell counts. Biomarker analyses focused on molecular type 2 activity status in biopsy specimens as they relate to asthma characteristics and clinical biomarkers.

Definition of type 2 status in biopsy specimens

Type 2–high versus type 2–low phenotypes were defined *a priori* based on inferred IL-13 activity in airway mucosal biopsy specimens from asthmatic patients compared with those from healthy control subjects by using gene expression of CCL26, periostin, or a multigene in-house IL-13–IVS (see the Methods section and Table E1 in this article's Online Repository at www.jacionline.org). IL-13 activity was selected because it is the most broadly expressed type 2 cytokine produced by T_H2 cells[20] and also type 2 innate lymphoid cells.[21] CCL26 mediates eosinophil infiltration into the airway and is the most highly expressed selective IL-13–driven gene.[22-24] The chemokine CCL17 is induced by IL-13[24,25] and decreased by anti–IL-13 and anti–IL-4 receptor mAb therapeutics.[10,13,26] For CCL26, the highest signals in the healthy control subjects were just below the limit of reliable quantification for the microarray, and therefore this limit of quantification (log_2 intensity of 5.0) was set as the threshold for airway CCL26–high versus airway CCL26–low status. For periostin and IL-13–IVS, type 2–high status was defined as gene expression (or enrichment) beyond the 95th percentile of the healthy control subjects.

Clinical biomarkers evaluated for their association with type 2–high or type 2–low status by airway IL-13–driven gene expression included FENO values, bEOS counts, spEOS counts, serum IgE levels, and expression of 2 serum type 2–associated biomarkers, serum CCL17 (sCCL17) and serum CCL26 (sCCL26). Details of the assays can be found in the Methods section in this article's Online Repository. Expression of sCCL17 and sCCL26 was measured repeatedly over 12 months to assess stability.

Statistical analysis

Healthy subjects (n = 25) and non–ICS-treated patients with mild asthma (n = 28) were analyzed separately, whereas the ICS-treated moderate (n = 29) and severe (n = 26) asthma cohorts were pooled. We focused analyses on the patients with moderate-to-severe asthma because they have unmet clinical need despite ICS treatment. Separate analyses on the moderate and severe asthma cohorts were not undertaken because of limited statistical power.

Statistical analyses used Array Studio (version 7; OmicSoft, Cary, NC; www.omicsoft.com). For data with log-normal distributions, logarithmic transformations were performed. Group comparisons of gene and protein expression levels (log_2-transformed) were performed by using general linear