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Journal

Annals of the American Thoracic Society, 10(Supplement)

ISSN

2329-6933

Author

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Publication Date

2013-12-01

DOI

10.1513/annalsats.201303-070aw

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Subtypes of Asthma Defined by Epithelial Cell Expression of Messenger RNA and MicroRNA

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Abstract

Human asthma can be subcategorized in several ways, but one powerful approach is to subtype asthma on the basis of underlying cellular and molecular mechanisms. Groups of patients with a disease that share a common underlying biology are termed an "endotype." Endotypes of asthma have been studied at both the cellular level (by cytological examination of induced sputum) and, increasingly, at the molecular level. Genome-wide analyses of mRNA expression within the lung have been useful in the identification of molecular endotypes of asthma and point to protein biomarkers of those endotypes that can be measured in the blood. More recently, studies of microRNA expression in airway epithelial cells in asthma have identified additional candidate biomarkers of asthma endotypes. One potentially valuable property of microRNAs is that they can also be measured in extracellular fluids and therefore have the potential to serve directly as noninvasively measured biomarkers.

Keywords: endotype; biomarkers; airway epithelium

(Received in original form March 28, 2013; accepted in final form June 10, 2013)

Supported by the NIH (HL095372, HL097591, HL108596), a research grant from Genentech, and the UCSF Sandler Asthma Basic Research (SABRE) Center.

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Ann Am Thorac Soc Vol 10, Supplement, pp S186–S189, Dec 2013
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DOI: 10.1513/AnnalsATS.201303-070AW
Internet address: www.atsjournals.org

A disease endotype is defined as a "subtype of disease defined functionally and pathologically by a molecular mechanism or by treatment response" (1). Endotypes, therefore, differ from phenotypes in that endotypes refer to specific groups of people (rather than their characteristics) and demand that these groups share some common underlying biology. One great value of identifying endotypes of diseases is that these endotypes point to specific therapeutic approaches. There are, of course, several challenges in implementing these therapeutic approaches even after endotyping, including the development of effective and specific therapeutic blockers for the pathways of interest, and developing noninvasive biomarkers that identify people who comprise the endotype.

In asthma, inflammation and airway obstruction are triggered by allergen exposure (2) through several intermediary steps that have been extensively studied in murine models. These steps include the production of "epithelial-derived cytokines," including thymic stromal lymphopoietin, IL-33, and IL-25. Thymic stromal lymphopoietin promotes the influx of dendritic cells, which present antigen to T cells, and IL-33 and IL-25 promote IL-13 and IL-5 release from group 2 innate lymphoid cells in the airway (3). Ultimately, these processes lead to helper T type 2 (Th2) cell differentiation and further production of the Th2 cytokines IL-4, IL-5, and IL-13 (4–6), and memory T and B cells are generated that can lead to chronic inflammation. Much of the remodeling and dysfunction in resident lung cells that are associated with asthma can be reproduced by IL-13 exposure, including goblet cell metaplasia (7), subepithelial fibrosis (8), and airway hyperresponsiveness (9–12). IL-4 can mimic many of the effects of IL-13 and is thought particularly important in IgE class switching by B cells. IL-5 is an important mediator of eosinophil recruitment.

Although mouse models of asthma have allowed significant progress in understanding the molecular and cellular underpinnings of allergic airway disease, human asthma is a clinically heterogeneous disease (13, 14), and this clinical heterogeneity may reflect differences in underlying biology and point to specific endotypes (14, 15). Specifically, the Th2-driven pathways observed in these murine models of asthma reflect the molecular events occurring in only a subset of patients with asthma. Other subsets of patients with asthma seem to have no or relatively low-level activation of these biological pathways. This biological heterogeneity has been observed for many years and one classification system involves the

cytological analysis of induced sputum, dividing subjects as having (*1*) eosinophilic, (*2*) neutrophilic, (*3*) mixed eosinophilic and neutrophilic, and (*4*) paucigranulocytic asthma (16–18). Importantly, noneosinophilic asthma has a poor response to inhaled corticosteroids (19, 20) and is pathologically distinct with fewer mast cells, and less subepithelial fibrosis (21). Sputum eosinophilia has also guided the use of mepolizumab, a monoclonal antibody that blocks IL-5, a key mediator in the differentiation, recruitment, and activation of eosinophils (22). Although early clinical trials of mepolizumab in relatively unselected patients with asthma were unsuccessful, the results of clinical trials of mepolizumab improved with patient selection based on the presence of sputum eosinophilia (23, 24). Similarly, one early clinical study of a therapeutic that targets the IL-4 receptor (thereby blocking the effects of both IL-4 and IL-13) did not show clinical efficacy in unselected patients with asthma (25). However, a phase 2 trial of a monoclonal antibody targeting the IL-4 receptor now reports efficacy in patients with asthma who were selected on the basis of peripheral blood or sputum eosinophilia (26). These data highlight the great value of classifying subjects with asthma into cytological endotypes when targeted therapies are employed. These data also highlight the need for a better understanding of patients with asthma who have a paucity of Th2 inflammation and the need to identify appropriate therapeutic approaches in this subgroup if conventional antiinflammatory therapies are ultimately shown to be ineffective or significantly less effective in this patient population.

Airway Epithelial mRNAs as Endotypic Markers

Given the success in endotyping based on induced sputum cytology, and the observation that emerging biological therapies in asthma typically target single cytokines or inflammatory pathways, one can expect some advantages to characterizing asthma endotypes at the molecular level, based on the activity of specific cytokine pathways. Because accurate measurement of cytokines in lung samples can be challenging, we have taken the approach of studying airway epithelial brushings obtained bronchoscopically as a "sensor" for inflammatory events in the airway (27, 28). Among the most highly induced genes in patients with mild asthma as compared with healthy control subjects, we found three IL-13– and IL-4–regulated genes: *chloride channel, calcium-activated, family member 1* (*CLCA1*); *periostin*; and *serine peptidase inhibitor, clade B* (*ovalbumin*), *member 2* (*serpinB2*, also known as *plasminogen activator inhibitor-2*). This set of three genes was induced in a subset of our subjects with asthma, and those subjects had increased expression of IL-13 and IL-4 by quantitative PCR in accompanying bronchial biopsies, and increased eosinophilia in bronchoalveolar lavage fluid. Therefore, we considered these three genes to be markers of a "Th2-high" endotype of asthma (14, 28), which is highly overlapping with "eosinophilic asthma" as described by cytological analyses of sputum.

In subsequent analyses, we found that subjects with Th2-high asthma, based on the expression levels of these genes, differed from subjects with Th2-low asthma with respect to physiology, lung inflammation, pathology, and response to inhaled corticosteroid therapy (28). For example, although both Th2-high and Th2-low subjects had airway hyperresponsiveness (as measured by the PC_{20} methacholine, i.e., the concentration of methacholine required to effect a 20% decline in FEV_1), Th2-high subjects had, on average, greater airway hyperresponsiveness. Significant bronchoalveolar lavage eosinophilia was restricted to subjects in the Th2-high group. Th2-high subjects also had increased numbers of intraepithelial mast cells (29) and increased subepithelial fibrosis (28). Finally, in a randomized placebo-controlled trial of inhaled corticosteroids, subjects with Th2-high asthma showed improvements in FEV_1 to inhaled corticosteroids, whereas the Th2-low subgroup did not (28).

Ultimately, the measurement of these three marker genes in bronchoscopically obtained airway epithelial brushings is a valuable research tool, but is not useful for stratification of patients in the clinic. On the basis of these data, and the observation that periostin is secreted by airway epithelial cells (30), blood levels of periostin have been studied as a surrogate marker for airway eosinophilia (31) and as a method for predicting response to pharmacologic IL-13 blockade with lebrikizumab, and anti–IL-13 antibody (32).

Airway Epithelial MicroRNAs as Endotypic Markers

More recently we have observed that microRNAs (miRNAs) are also highly differentially expressed in the airway epithelium of subjects with asthma as compared with healthy control subjects (33). miRNAs are approximately 22-nucleotide RNAs that promote mRNA degradation (34) or inhibit translation (35), by binding with at least partial complementarity to the 3′ untranslated region of target mRNAs. miRNAs have been implicated in the regulation of fundamental biological processes in epithelial cells such as cell proliferation, differentiation, and apoptosis (36–38). One focus of our ongoing work is to study whether these miRNAs regulate asthma-relevant biological functions of these epithelial cells. However, one might also ask whether these miRNAs can serve as biomarkers of asthma endotypes. Using a similar approach to that which we employed for assessment of mRNA expression in airway epithelial cells, we performed companion experiments with bronchial epithelial cells *in vitro* and found that IL-13 had significant effects on bronchial epithelial miRNA expression and that several of these changes recapitulated the differences between asthma and health that we observed in our human studies (33). One finding that we have focused on in subsequent work is consistent repression of four members of the miR-34/449 family (miR-34c-5p, miR-34c-5p, miR-449a, and miR-449b-5p) both *in vivo* in asthma and *in vitro* by IL-13. One study found that miR-449 regulates the differentiation of ciliated epithelial cells, in part by targeting *NOTCH1* mRNA (39). These data provide some clues as the potential biological role of the miR-34/449 family in airway epithelial cells.

Whether these miRNAs, or others that are regulated by IL-13, can serve as markers of a Th2-high asthma endotype is uncertain; however, miRNAs have a relatively unique property that renders them valuable as biomarkers. That is, miRNAs can exist in extracellular fluids in

forms that are resistant to degradation by RNases, and therefore can be measured in sputum, bronchoalveolar lavage fluid, and blood using PCR, microarrays, and sequencing methods. There are at least three ways that miRNAs are protected from RNases. First, miRNAs can be contained within membrane-bound vesicles termed "exosomes" (40). Second, miRNAs can be bound by proteins such as Argonaute (41). Finally, miRNAs can be protected by residence within high-density lipoproteins (42). Consequently, if specific miRNAs are differentially expressed by airway epithelial cells under the influence of specific cytokines (as in the data described previously) or by disease-relevant inflammatory cells, and are then secreted into the airway surface liquid or into the blood, these miRNAs could themselves be used as biomarkers of asthma endotypes related to these specific cytokines and inflammatory cells. Other potential applications of these extracellular miRNAs as biomarkers could be to mark the type or degree of epithelial cell (or T-cell) differentiation that is occurring in any specific patient.

A series of mouse studies have been performed that identify miRNAs that regulate various aspects of allergic inflammatory responses and that could, therefore, serve both as biomarkers in human asthma and as therapeutic targets. For example, miR-126 suppresses the effector function of Th2 cells and the development of allergic airway disease in a mouse model of house dust mite–induced allergic airway disease (43). The same mouse–house dust mite exposure model is associated with increased expression of miR-145, miR-21, and let-7b in the airways (44). miR-21 is up-regulated in mouse models of allergic airway inflammation and regulates expression of IL-12p35 (45), an important Th1 cytokine. miR-181a, miR-146a, and miR-146b are expressed in spleen $CD4^+$ T lymphocytes and appear to play proinflammatory roles in a murine model of asthma (46). miR-375 was found to be down-regulated in IL-13 transgenic mice and repressed in human bronchial (and esophageal) epithelial cells by IL-13 (47). let-7 has a complicated but proinflammatory role in a murine model of asthma (48). Finally, broad-based miRNA profiling has been performed in murine models of acute and chronic asthma, identifying additional possible biomarkers (49). To date there have been few studies that relate blood miRNAs in human subjects to findings made in mouse models, but one study found that miR-221 and miR-485-3p are up-regulated in the blood of asthmatic compared with healthy children and are also up-regulated in an ovalbumin-induced mouse model of asthma (50).

Conclusions

The classification of patients with asthma into specific endotypes has the potential to improve asthma therapy by guiding the targeted use of specific novel therapies such as those that block specific cytokines. In addition, it is possible that endotyping will be useful in the application of widely used existing antiinflammatory therapies such as inhaled corticosteroids and leukotriene pathway inhibitors. However, all of these applications will require the development and refinement of noninvasively measured biomarkers that can accurately mark the underlying lung endotype, and appropriate clinical studies. One such candidate biomarker is periostin, which was developed on the basis of mRNA studies of bronchoscopically obtained airway epithelial brushings. Similar data now exist for miRNAs that are abnormally expressed in the airway epithelium in asthma and that have the potential to mark specific endotypes. One interesting property of miRNAs is that they are protected from RNases and can be measured in extracellular fluids. Therefore, one approach to the development of biomarkers is the measurement of these miRNAs directly in blood and/or sputum. ■

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

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