

Dissecting Asthma Transcriptomics: Does Site Matter?

Asthma is a clinically and pathologically diverse chronic airway disease, affecting >200 million people worldwide (1). Importantly, 5–10% of patients suffer from severe disease that is insensitive to conventional treatment with corticosteroids and requires high-dose inhaled corticosteroids plus systemic corticosteroids and/or a second controller therapy (2). These patients account for a disproportionate amount of total asthma healthcare resources, and represent an urgent unmet clinical need. To develop novel and more targeted therapies, we need to improve our understanding of the molecular mechanisms that drive asthma pathogenesis. However, this remains a challenge due to the marked heterogeneity among asthma phenotypes. Given the prominent role of T cells in the pathophysiology of asthma, specific T-cell signatures have been used to broadly stratify asthma subphenotypes into T-helper cell type 2 (Th2)-high, Th2-low, and, more recently, Th17-high groups (3, 4). Most previous genomic studies in this area have focused on profiling peripheral blood samples or mixed airway cell populations from epithelial brushings, sputum, or biopsies (5–7). In contrast, airway T cells are relatively understudied, and comprehensive transcriptomic analyses of airway T cells are lacking. Furthermore, personalized therapeutic strategies targeting Th2 and Th17 pathways have produced mixed results (2), highlighting unknown complexities of asthma pathogenesis that cannot be explained by Th cell phenotypes alone, but rather arise through interactions between multiple cell types and effector pathways.

In this issue of the *Journal*, Singhania and colleagues (pp. 261–270) report on the transcriptomic profiles of CD3⁺ T cells purified from sputum or BAL, alongside epithelial brushings from 46 subjects with asthma and 19 healthy controls (8) (Figure 1). Their study included participants across a range of asthma severities: 15 mild asthmatics on β_2 -agonists alone, 17 moderate asthmatics on inhaled corticosteroids, and 14 severe asthmatics on high-dose inhaled corticosteroids. The authors sought to identify gene-expression patterns associated with asthma severity, and explore interactions between airway-derived T cells and the epithelium.

Overall, comparisons between severe asthmatics and healthy controls yielded the largest number of differentially expressed genes, primarily in sputum CD3⁺ T cells and to a lesser extent in the epithelium, suggesting that the impact of high-dose inhaled corticosteroid treatment may be tissue-type dependent. Fewer genes were found to be differentially expressed between mild/moderate asthmatics and healthy controls across these cell types. Upregulation of IL-13-responsive genes, mast cell markers, and inducible nitric oxide synthase was observed exclusively in the epithelium of mild asthmatics (who were not taking corticosteroids). These findings suggest that treatment with inhaled corticosteroids effectively dampened the Th2 inflammatory response in the epithelium of moderate asthmatics. In contrast, the gene-expression profiles of CD3⁺ T cells from BAL were relatively

unaffected by asthma status. The authors postulate that this may reflect the relatively minor impact of disease on this compartment, as BAL typically samples the distal airway.

Singhania and colleagues then performed a network analysis to unveil molecular interactions between the epithelium and T cells. Network analysis is based on the concept that a functioning biological system can be represented as a network graph of interacting gene products. Here, the authors extended this approach to infer protein–protein interactions operating within and between T cells and epithelial cells. A fundamental organizing principle of biological networks is their nonuniform architecture: most proteins have few interactions, and a few proteins (designated as “hubs”) have many interactions (9). The most highly interconnected genes within the network included several molecules associated with Toll-like receptor (TLR) signaling and innate immunity, but these signals were derived from T cells rather than epithelial cells. Of note, the authors demonstrated surface expression of CD14 and TLR2 in peripheral blood T cell subsets, with the highest expression on the innate-like mucosal associated invariant T (MAIT) cell population. Key genes that were upregulated in the epithelium of severe asthmatics included 5-aminolevulinate synthase (*ALAS2*), carcinoembryonic antigen-related cell adhesion molecule 5 (*CEACAM5*), mucin 5AC (*MUC5AC*), pleckstrin homology-like domain family B member 2 (*PHLDB2*), synuclein α (*SNCA*), transcobalamin 1 (*TCN1*), and transferrin. The prominence of innate immune mechanisms identified in these analyses and the detection of the neutrophil granule constituent *TCN1* may also reflect the high proportion of neutrophilic severe asthma in their cohort. Specifically, upregulation of *CEACAM5* within the epithelium is in line with reports of increased epithelial expression of other CEACAM family molecules in severe neutrophilic asthma (10). Replication of these findings in a larger cohort with greater representation of other severe asthmatic phenotypes (eosinophilic or mixed neutrophilic/eosinophilic) would be of interest.

The authors also performed a targeted analysis of Th2 and Th17 gene signatures by assessing the relative gene expression of established markers of IL-13 and IL-17 induction across all sample types and asthma severities. Significant upregulation of Th2 signature genes was observed exclusively in the epithelium of mild asthmatics, whereas expression of Th17 signature genes increased with asthma severity in sputum CD3⁺ T cells alone, and was highest in those from severe asthmatics. This is consistent with previous reports of a steroid-insensitive Th17 pathway underlying severe asthma, and again potentially relates to the pathophysiology of neutrophilic asthma specifically, as IL-17 secretion by Th17 cells has been implicated in the induction of neutrophilic airway inflammation (11).

It is important to acknowledge that this is an observational study, and therefore it is not possible to differentiate between mechanisms that are driving disease and those that are reacting to

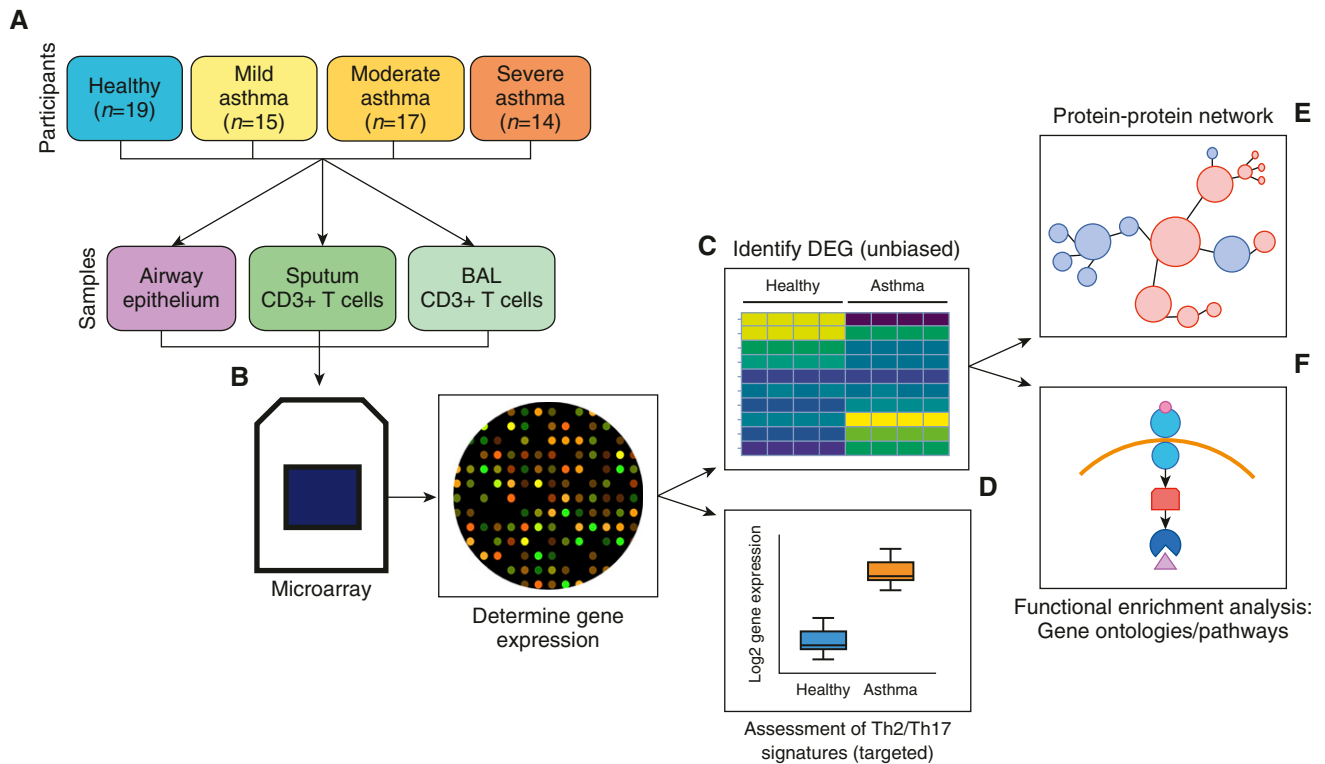


Figure 1. Workflow of the methodology and analysis. (A) Airway epithelium and CD3⁺ T cells were isolated from the study participants. (B) Gene-expression profiles were determined by microarray profiling. (C) Differentially expressed genes (DEG) were identified by comparing each asthmatic severity group with healthy controls, across all sample types, alongside (D) targeted analysis of T-helper cell type 2 (Th2) and Th17 gene signatures. (E) A protein-protein interaction network was constructed from the differentially expressed genes. (F) Enrichment analysis was performed to identify relevant biological pathways and functions.

disease. Another limitation is that most of the subjects with asthma also had atopy, and these subjects were compared with a control group of healthy subjects without atopy. Thus, the findings will reflect mechanisms that are associated with both atopy and asthma. Another key question concerns the relative contributions of different T cell subtypes to the observed gene-expression changes in sputum. This is important given the known phenotypic and functional heterogeneity of T-cell subsets, and the unknown role of innate-like MAIT cells. Interestingly, the authors previously reported a significant reduction in the frequency of sputum CD3⁺ MAIT cells in severe asthmatics compared with healthy controls (12). Detailed mechanistic studies will be required to dissect the role of MAIT cells in this disease.

Given the differences the authors observed between sputum and BAL T cells, future studies should aim to examine T cells from collagenase-dispersed bronchial biopsies, which are arguably the most clinically relevant samples. This was attempted by the authors, but the yields were too low for genomic analysis. This is a common problem encountered in studies of this nature, but it can be circumvented by the use of new technologies such as single-cell RNA sequencing (13). Another area for future development would be to more comprehensively investigate the mechanisms that mediate cross-talk between epithelial cells and T cells. The authors used gene-expression profiles from airway epithelial cells and T cells to infer these mechanisms; however, their analyses focused on differentially expressed genes, which were limited in number. An alternative approach would be to leverage the genomic profiles of

each cell type to build a comprehensive network of all expressed ligands and corresponding receptor pairs (14). Notwithstanding these limitations, this study presents a novel approach for investigating the mechanisms underlying severe asthma. This work illustrates that disease-driven gene-expression profiles are distinct across tissue types and airway compartments, as reported by others (15). The multitissue transcriptomics approach coupled with a cross-tissue network analysis has strong potential to further our understanding of the mechanisms underlying the subphenotypes of severe asthma, and to unveil novel opportunities for therapeutic intervention. ■

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

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