IFN-stimulated Genes and Non-Type 2 Inflammation New Pathway or Red Herring?

Asthma is a heterogeneous disease comprised of numerous complex disease endotypes with different underlying pathophysiologic mechanisms, natural histories, and treatment responses. Previous gene-expression studies in airway epithelium demonstrated an enrichment of type 2 (T2) inflammatory genes in the airways of subjects with mild asthma, and identified that these genes are associated with increased airway responsiveness, IgE levels, and eosinophilic inflammation (1, 2). T2 genes are integral to the development of several asthma endotypes and are the targets of new biologic agents for severe asthma (1). There is, however, growing evidence that roughly half of individuals with asthma have non-T2-mediated airway inflammation and thus do not benefit from inhaled corticosteroids (ICS) or the new biologic agents (2–4). Although sputum neutrophils may be increased in these individuals (3, 5), such an increase is not found in all non-T2 asthma endotypes, underscoring the fact that our understanding of the non-T2 inflammatory mechanisms that differentiate these endotypes is limited.

In this issue of the *Journal*, Bhakta and colleagues (pp. 313–324) report their analysis of the relationship between IFN-stimulated genes (ISGs) and T2 inflammation, and describe the impact of ISGs on lung function and bronchodilator response in subjects with mild asthma (6). Using RNA-sequencing data from airway epithelial cells of subjects with mild asthma and healthy control subjects, the authors confirmed previous analyses in which differential expression of known T2 genes was identified. In addition, using ingenuity pathway analysis to elucidate the upstream regulators of the overrepresented genes, the authors identified a set of ISGs that were enriched in the airways of subjects with asthma. Their ISG signature was differentially expressed both in the airway epithelium of subjects with mild asthma compared with healthy control subjects and in cultured human bronchial epithelial cells after IFN- α stimulation. The authors demonstrated that higher ISG expression was associated with reduced FEV₁ and increased bronchodilator response in subjects with mild asthma and that these associations were independent of T2 inflammation. Furthermore, the association of ISG with decreased FEV₁ and ISG expression overall was attenuated by ICS treatment. However, the ISG signature in the whole blood of individuals with both mild and severe asthma was not associated with FEV₁ or treatment with corticosteroids.

This study has several strengths that make it a significant addition to the rapidly growing literature on non-T2 pathways in asthma. First, it strengthens the argument that T-helper cell type 1 (Th1) pathways, including ISGs, are important regulators of non-T2 inflammation in individuals with mild asthma. The results of the current work extend previous investigations in mouse models that showed that IFN- γ is associated with increased airway responsiveness (7). Human studies have also shown increased

IFN- γ expression in bronchial biopsy specimens from subjects with atopic asthma compared with control subjects (8) and a higher percentage of CD4⁺ T cells, increased IFN- γ expression, and higher IFN- γ protein levels in the BAL fluid of subjects with severe asthma compared with subjects with mild to moderate asthma (7).

The results of this study also provide further evidence that T1 pathways can influence asthma pathogenesis independently of T2 inflammation, and are similar to recent findings from the Severe Asthma Research Program. Using weighted gene coexpression network analysis in airway epithelium from subjects with severe asthma, they identified gene-expression modules that were associated with T1 inflammation and IFN genes, but were not associated with T2 inflammation or asthma severity. Only 21% of the subjects with upregulation of T1 gene expression had concomitant upregulation of T2 genes. Furthermore, the individuals who were identified as T1-high or T2-low based on this weighted gene coexpression network analysis were the youngest subjects, had the lowest fractional exhaled nitric oxide and highest quality-of-life scores, and represent a distinct asthma endotype (9).

In the current study, the authors also present evidence that endoplasmic reticulum (ER) stress is increased in the airways of individuals with asthma, is associated with both the ISG signature and T2 inflammation, and decreases with ICS treatment. These findings add to previous work that demonstrated increased ER stress in subjects with asthma (10) and in animal models upon ovalbumin challenge (11, 12). Importantly, suppression of ER stress was shown to attenuate airway inflammation, mucus production, and bronchial hyperresponsiveness in a mouse model of allergic airway disease (11, 12), supporting the notion that ER stress may be a new therapeutic target in asthma.

Several limitations to the current study are worthy of mention. The most notable limitation is the small sample size used for the initial RNA sequencing. The sample size limits not only the power to detect differential expression but also the generalizability of these findings to subjects with more severe disease. It is concerning that the ISG signature was equally upregulated in both mild and severe asthma, which suggests that ISGs are unrelated to asthma severity. This discrepancy raises the possibility that this mechanistic association is the result of unmeasured confounders in a small cross-sectional study. Furthermore, the small sample size impacted the authors' ability to investigate the role of the viral genome in ISGs. Although they did not identify an association between viral genomes and upregulation of ISGs, the fact that they identified only a small number of viral genomes, and only in control subjects, limits our ability to draw meaningful conclusions from these data. Therefore, the lack of association between the viral genome and ISGs should be interpreted with caution.

Although the ingenuity pathway analysis identified IFNs as important upstream regulators of a gene set that was found to be differentially expressed between subjects with asthma and control subjects, the fact that the IFNs themselves and other

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important genes in the pathway were not identified as being differentially expressed raises the concerning possibility of additional, unmeasured confounders in the analysis, which are unlikely to be explained by the nature of the sequencing platform.

Although the results of this study provide proof-of-concept evidence for a mechanistic link between ISGs and ER stress and asthma, additional investigations are needed to fully elucidate the biological basis of these associations. Importantly, these results should motivate further studies of the impact of ISGs on asthma phenotypes and the role of ER stress in asthma pathogenesis. ■

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Impact of Chronic Obstructive Pulmonary Disease on Immune-based Treatment for Lung Cancer Moving toward Disease Interception

Recent groundbreaking studies reveal responses in approximately 20% of patients with non-small cell lung cancer (NSCLC) treated with inhibitors of the PD-1/PD-L1 (programmed death-1/programmed death-ligand 1) checkpoint. This includes robust and durable responses in previously treated patients with progressive locally advanced or metastatic NSCLC, as well as significant antitumor responses in treatment-naïve advanced disease (1, 2).

NSCLCs, often carcinogen induced, have among the highest mutational loads of all malignancies (3). Nonsynonymous mutations can give rise to mutant proteins that, when processed, may result in immunogenic peptides, defined as neoepitopes that avidly bind and are presented in the context of autologous major histocompatibility complex molecules (4). The

recently documented clinical efficacy of checkpoint blockade immunotherapies in NSCLC and other malignancies indicates that tumor-specific T cells can recognize neoantigens, resulting in tumor cell death. Could this also be consistent with the theory of immunosurveillance? The concept suggests that the host immune response is able to recognize and prevent the outgrowth of invasive cancer cells at the earliest point of development and thus before clinical recognition. The theory of immunosurveillance has been more recently refined to include the concept of immunoediting, which encompasses the dynamic continuum of the interplay between the tumor and immune system (5, 6). Cancer immunoediting refers to the protective immune response, which limits tumor growth but also shapes the character of the developing tumor. A central tenet of cancer immunoediting is that T-cell recognition of tumor antigens drives the immunological elimination or shaping of a developing cancer.

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