The ABCs and DEGs (Differentially Expressed Genes) of Airway Hyperresponsiveness

Airway hyperresponsiveness (AHR) is a defining feature of asthma, wherein airway responses to various inhaled stimuli exhibit increased sensitivity by reacting to a lower dose, and increased reactivity by developing a greater magnitude of response to a given dose. Accordingly, the dose–response curve shifts leftward, with progressive loss of the response plateau relative to the nonasthmatic airway; the greater the degree of AHR, the greater the shift and magnitude of the response. Stimuli have been classified as selective and nonselective. Selective stimuli are immunologic, such as allergens, or nonimmunologic, such as aspirin. Nonselective stimuli can either be direct-acting agents that interact with specific airway smooth muscle receptors to activate intracellular mechanisms of bronchoconstriction, such as histamine on H_1 receptors or methacholine on muscarinic M_3 receptors, or indirect by acting through one or more intermediate pathways (cold air/exercise, mannitol, adenosine monophosphate), causing effector cells to release inflammatory mediators that cause bronchospasm.

The occurrence of AHR has been appreciated for decades, and standardized methodologies to objectively measure AHR with both direct (1) and indirect stimuli (2), for both clinical (diagnostic) and research (mechanistic and therapeutic) purposes, have been developed. Despite our longstanding awareness of AHR, the precise mechanisms driving this phenomenon are poorly understood.

We recognize that AHR can be persistent or variable (3). Persistent AHR relates to structural changes within the airways that follow tissue remodeling mechanisms, which, by virtue of a reduction in airway lumen diameter, explain at least in part that component of AHR that does not resolve (4, 5). Airway smooth muscle cell size, number, and/or changes in contractile properties (6) as well as neural pathways (7) may also contribute to mechanisms of AHR. Variable AHR is associated with acute airway inflammation, such as the allergen-induced increase in AHR to methacholine that occurs with eosinophilic or T2 airway inflammation observed in patients with allergic asthma with dual responses after allergen exposure (8) or after viral infection and non-T2 airway inflammation (9). Variable AHR is partially improved with antiinflammatory treatment, such as inhaled glucocorticosteroids (ICS), further supporting a role for airway inflammation in driving AHR (8).

With respect to variable AHR and the role of airway inflammation, we read with interest the recent work of Murphy and colleagues (pp. 1565–1575) published in this issue of the *Journal* (10). Here, the authors report 120 differentially expressed genes (DEGs) in bronchial brushings of patients with asthma exhibiting AHR to both methacholine and exercise (i.e., both direct and indirect stimuli) versus patients with asthma exhibiting AHR to methacholine only. Fifteen of these DEGs, including mast cell–related genes (*KIT*, *CPA3*) as well as genes associated with T2 inflammation (*IL1RL1*, *CLCA1*, *POSTN*, *SERPINB2*, *NOS2*), were further analyzed for their relationship with patient characteristics, including lung function, response to direct and indirect stimuli, sputum T2 inflammatory profile, and airway cellular composition. Most (12 of 15) showed increased expression with indirect AHR, and all positively correlated with the density of mast cells and eosinophils in either airway wall epithelium or subepithelium. Notably, *IL1RL1* expression was positively correlated with mast cells in the epithelial compartment, and *IL1RL1* and *IL18R1* were both positively correlated with the density of intraepithelial eosinophils.

Further modeling using cells from asthmatic and non-asthmatic donors or from donors cell lines investigated how airway epithelial cells work together with mast cells and eosinophils to coordinate both T2 and non-T2 airway inflammation. *Ex vivo* coculture of airway epithelial cells with mast cells demonstrated that the epithelium promotes basal T2 gene expression in mast cells together with an epithelial-dependent increase in T2 gene expression after treatment with IL-33, and this inflammatory gene expression was only partially suppressed by glucocorticoids. Coculture of eosinophils with airway epithelial cells demonstrated that stimulation with IL-33 and IL-18 induces *IL13* and *IFNG* gene expression in eosinophils, suggesting that interaction with airway epithelium may also induce eosinophils to release non-T2 cytokines in the airways. Taken together with previous work describing T2 and non-T2 inflammation in the asthmatic airways (11–13), these experiments suggest that airway epithelial cells regulate gene expression by mast cells and eosinophils to modulate both T2 and non-T2 mechanisms of airway inflammation in individuals with asthma exhibiting AHR to both methacholine and exercise.

Although a positive test for exercise-induced bronchoconstriction is highly specific for asthma, and there is confidence in the findings regarding DEGs in those with asthma and indirect AHR to exercise, ideally the additional cellular investigations would be performed on cells derived from the study participants in whom the DEGs were identified versus healthy donors (airway epithelial cells), cell lines (LAD2 mast cells), or potentially individuals without asthma or exercise-induced bronchoconstriction (eosinophils were sourced from individuals in whom asthma and/or rhinoconjunctivitis had been diagnosed). Particularly important in that regard is that the airway epithelium in patients with exercise-induced bronchoconstriction, and other lung disorders, is likely not "healthy"; therefore, *in vivo* application of the findings should be interpreted with caution.

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Nonetheless, the findings support the observed clinical effectiveness of both inhaled glucocorticosteroids (14) and leukotriene receptor antagonists (15) against exercise-induced bronchoconstriction and are thought provoking with respect to the concerted role of epithelial cells, mast cells, and eosinophils in driving T2 and non-T2 airway inflammation as mechanisms responsible for indirect AHR. The clinical implications of these data with respect to treating exercise-induced bronchoconstriction are unclear. ■

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CEACAM6: A Novel Marker of Chronic Obstructive Pulmonary Disease Susceptibility?

Chronic obstructive pulmonary disease (COPD) is a progressive disease typified by airflow obstruction, lung inflammation, and airway remodeling. COPD is a major global health burden and is predicted to be the leading cause of death worldwide by the year 2030. Current therapeutic strategies are sparse and largely ineffective (1, 2). Although cigarette smoke (CS) exposure is the primary risk factor for COPD development, only 25% of people who smoke develop COPD, highlighting the importance of predisposing genetic and epigenetic factors and of environmental exposures as key contributors to COPD pathogenesis (3–5). Long-term imbalances in oxidative stress and inflammation are believed to drive the airway remodeling and alveolar destruction observed in chronic bronchitis and emphysema (1). Likewise, extrapulmonary manifestations such as cardiovascular disease, osteoporosis, and skeletal muscle wasting are also linked to oxidative stress and inflammation. The uncovering of novel therapeutic strategies to treat patients with COPD depends on understanding how these dysfunctions mechanistically support the pathogenesis of COPD.

Increases in cellular nitric oxide, induced by inflammation, result in a type of oxidative stress known as nitrosative stress, which is marked by increases in 3-nitrotyrosine (3-NT) and nitrogen dioxide.

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