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Glucocorticoids and Airway Smooth Muscle: A Few More Answers, Still More Questions

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Glucocorticoids (oral and inhaled corticosteroids) have been critical for the management of asthma symptoms owing to their exceptional therapeutic efficacy in mitigating airway inflammation. Glucocorticoids exert therapeutic effects in asthma primarily via their genomic actions in resident or invading lung cells (1), either by negatively regulating the expression of genes that promote inflammation or by inducing genes that inhibit inflammation. Although inhalation of corticosteroids provides efficient control of exacerbations in a large number of patients with asthma, corticosteroids have limited efficacy in many patients with asthma whose disease is severe or refractory (steroid-resistant asthma) (1). Multiple mechanisms have been proposed to explain the compromised glucocorticoid response in distinct cell types associated with asthma pathology, but the precise molecular mechanisms remain ill defined.

To date, most studies have emphasized immune cells and epithelial cells as the key targets of glucocorticoid actions in the asthmatic lung. However, glucocorticoids have potentially important immunomodulatory effects on airway smooth muscle (ASM) cells. Specifically, glucocorticoid actions in ASM can affect both airway inflammation (i.e., the immunomodulatory role of ASM) and airway remodeling (2–6). Glucocorticoids can also regulate the contractile function of ASM by *1*) reducing cholinergic hypersensitivity in the airways, possibly through regulation of the expression of G protein–coupled receptors (muscarinic: M_2, M_3; and histamine: H_1) that mediate airway contraction (7–9), or *2*) augmenting airway relaxation through upregulation of β_2AR (β_2 adrenergic receptor) and modulation of adenylyl cyclase activity (10, 11). Although studies over the last decade have provided significant insight into the complex molecular interactions of glucocorticoids in ASM cells, whether ASM from individuals with asthma responds differently to glucocorticoids is unknown, as is the contribution of this response to the phenomenon of steroid resistance in individuals with asthma.

In this issue of the *Journal*, Kan and colleagues (pp. 110–120) take an important step toward addressing this problem by characterizing the glucocorticoid-regulated transcriptome of ASM derived from patients with severe asthma (12). Specifically, the authors compare differences in mRNA abundance in ASM cells derived from donors with fatal asthma and donors without asthma, treated or not treated with budesonide. Additionally, the authors compare the dataset generated in this experiment with the transcriptomic profile of glucocorticoid response in other cell types (from a publicly available database), and identify signatures unique to ASM.

Several interesting observations are reported. Somewhat surprisingly, the transcriptome profiles were highly similar in ASM from donors with asthma and those without asthma, both at baseline and after stimulation with budesonide. In ASM cells from both donor cohorts, glucocorticoid-regulated genes such as *TSC22D3*, *CRISPLD2*, and *KLF15* were significantly upregulated, which is consistent with previous studies that examined glucocorticoid responses in bronchial biopsies and isolated ASM cells (13–15). Similarly, *FKBP5*, which has been previously reported as a negative regulator of glucocorticoid response in individuals with asthma, was also significantly upregulated (13). Two genes, *CCK* (cholecystokinin) and *PMEL* (premelanosome protein), were differentially expressed between the cohorts with and without asthma. The functions of these genes in ASM are poorly defined, and additional studies focused on examining their role in rendering ASM cells glucocorticoid resistant are essential to establish their physiological relevance in the development of steroid resistance in the disease state.

Although the paper by Kan and colleagues is a critical step toward identifying glucocorticoid-regulated molecular networks that are differentially regulated in ASM cells from patients with severe asthma, the clinical significance of these observations is unclear and remains to be determined in future work. As duly noted by the authors, the ASM cells were derived from postmortem specimens with cursory background information lacking specific clinical data or comments on steroid usage and resistance, if any. It is also essential to note here that isolated ASM cells retain molecular and physiological traits that are reflective of their behavior *in vivo* and have been used extensively in basic and translational research. Understandably, the ASM cells used in the current study were isolated from tracheae or large bronchi. However, ASM cells from smaller airways play a crucial role in influencing airway inflammation, remodeling, and resistance, and may respond differently to glucocorticoids and be equally or more important than large-airway ASM cells in contributing to steroid resistance in patients with asthma. Finally, a validation of these targets using a secondary approach (such as real-time qPCR) or biochemical analysis of proteins is also lacking. Notwithstanding these limitations, the current effort by Kan and colleagues shows that glucocorticoid responses are altered in ASM cells from individuals with severe asthma.

Understanding the mechanisms that contribute to steroid resistance in patients with asthma is a profound challenge that is made more difficult not only by the complexity of the disease asthma *per se* but also by the fact that multiple cell types contribute to asthma pathobiology, and the function of each of these cells is regulated by glucocorticoids. Although the clinical effect of steroid treatment can be readily assessed, the success or failure of steroid treatment relies on the sum of the effects of the drug on multiple cells, and how steroids impact the singular and cooperative actions

of the cells that cause asthma. Given that it is extremely difficult to decipher the relative contributions of individual cell types to drug responses in integrative human studies (i.e., most clinical research), and despite the inherent limitations of *in vitro* studies (16), a logical approach to understanding these mechanisms is to pursue more reductionist studies in individual cell types and then attempt to piece data from such studies into a understanding of the gestalt. Accordingly, computational biology methods to analyze transcriptome data (and other omics-based approaches) along with extensive clinical and patient phenotype data would greatly serve this purpose and clearly represent an important path for future research.

Careful consideration of the limitations of reductionist/*in vitro* studies is necessary to inform study design and enable interpretation. Relevant to Kan and colleagues, the source of cells from patients with asthma is an important consideration. Cells obtained from ASM tissue collected after an autopsy have potential viability concerns, but perhaps more importantly, the ability to ultimately match experimental data with a patient's clinical profile or disease phenotype is often limited compared with what can achieved in prospective studies in which tissue can be procured from (live) patients. Yet, the latter situation also has limitations, in that tissue procurement occurs via bronchial biopsies, which are highly variable with respect to the amount and viability of ASM collected, and involve a fairly invasive (and expensive) protocol.

Despite these and other technical and design challenges of cell-based studies, studies such as those conducted by Kan and colleagues are critical to the slow inductive process of acquiring sufficient data to gain insight into the pathobiology of asthma and a patient's response to asthma drugs. Ultimately, the refinement of experimental designs coupled with better technology will grow the database and advance this field of study. ■

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