17. Mould KJ, Moore CM, McManus SA, McCubbrey AL, McClendon JD, Griesmer CL, *et al*. Airspace macrophages and monocytes exist in transcriptionally distinct subsets in healthy adults. *Am J Respir Crit Care Med* 2021;203:946–956.

18. Johansson C, Kirsebom FCM. Neutrophils in respiratory viral infections. *Mucosal Immunol* 2021;14:815–827.

Bronchial Epithelial Cell CC16 mRNA: Novel Asthma Biomarker or the Same Book with a New Cover?

Asthma is a heterogeneous disease with the involvement of T2 and non-T2 inflammatory pathways intersecting with a variety of environmental exposures and clinical phenotypes. Even within the group of patients with severe asthma with elevated T2 biomarkers, there is heterogeneity in mechanism and in the value of T2 biomarkers for predicting treatment response (1–3). Therefore, there remains an unmet need for the discovery of novel biomarkers in severe asthma from both a mechanistic and therapeutic perspective. In other words, we need to continue to strive toward the refinement of severe asthma endotypes. The examples of fractional exhaled nitric oxide and sputum eosinophil counts prompt continued exploration of airway-based biomarkers as a more direct indicator of airway biology compared with serum-based biomarkers. As we have come to understand the importance of the airway epithelium in the asthmatic response and now even have a biologic therapy (tezepelumab) directed toward an epithelial-derived cytokine (4), the airway epithelium has emerged as an attractive target for biomarker discovery. An epithelial-derived biomarker that has been studied in population studies is CC16, a secretory protein from club cells. In previous studies, largely of serum concentrations of CC16, low concentrations have been associated with accelerated lung function decline and increased airway hyperresponsiveness (5, 6).

In this issue of the *Journal*, Li and colleagues (pp. 438–451) published a study in which they used RNA sequencing and gene expression microarray data in bronchial epithelial cells (BECs) from the NHLBI (National Heart, Lung, and Blood Institute) SARP (Severe Asthma Research Program) to determine if CC16 mRNA concentrations are associated with asthma severity (7). In this study, CC16 mRNA expression levels in BECs and asthma-related phenotypes in the SARP population (242 patients with asthma and 69 nonsmoking control subjects) were analyzed, adjusting for age, sex, body mass index, race, and batch effect. Data were derived from both SARP longitudinal and cross-sectional cohort participants who had epithelial cell samples available from brush biopsies obtained via bronchoscopy.

CC16 mRNA expression levels in BECs were significantly lower in patients with severe asthma than in those with nonsevere asthma in the cross-sectional and longitudinal cohorts. Reduced CC16 mRNA expression levels in BECs were significantly associated with decreased prebronchodilator FEV_1% predicted and FEV_1/FVC. In both cohorts, reduced CC16 mRNA concentrations in BECs were significantly associated with increased fractional exhaled nitric oxide concentrations and sputum percent eosinophils. Among a subset of selected T2 gene transcripts (*IL1RL1*, *IL18R1*, *POSTN*, *SERPINB2*, *CLCA1*, *NOS2*, *MUC5AC*, and *PLA2G4A*), there was a negative correlation with CC16 mRNA concentrations, whereas CC16 mRNA expression levels in BECs were positively correlated with mRNA expression levels of Th1 pathway and inflammation genes (*IL12A*, *MUC5B*, *C3*, *TLR5*, and *CXCL6*). High CC16 mRNA expression levels in BECs were significantly associated with younger age, higher percent non-Hispanic White, and lower body mass index. The authors then subcategorized participants with asthma into four groups on the basis of the combination of BEC CC16 mRNA expression level and plasma IL-6 concentrations, which had been previously demonstrated by SARP to be associated with severe asthma independent of T2 status (8). For this subanalysis, the authors merged patients with asthma in the cross-sectional and longitudinal cohorts to increase sample size and power. They identified four endotypes with this combinatorial approach: T2 obese severe asthma (low CC16 and high IL-6), T2 nonobese severe asthma (low CC16 and low IL-6), non-T2 obese severe asthma (high CC16 and high IL-6), and non-T2 nonobese nonsevere asthma (high CC16 and low IL-6).

These results are novel, given the focus on gene expression in a specific cell type and airway epithelial cells, and provide further encouragement for continuing to pursue single-cell RNA sequencing approaches from airway specimens, not only for the study of mechanism but for the potential prediction of treatment response. Although the use of bronchoscopy brush samples limits the immediate practical translation of these data, the approach of the authors further highlights the imperative to find ways to refine airway sampling in translational asthma research with the eventual goal of bringing these methods to the clinical arena. Induced sputum is a potential solution, though it has been technically challenging to perform an in-depth analysis of single cells from induced sputum. However, recent advances in technology are starting to overcome these barriers (9).

There are several limitations of the study to consider. This study highlights the potential role of CC16 as a possible biomarker in severe asthma but does not elucidate the biology of the impact of CC16 on T2 inflammation. This will be an important future direction in better understanding the role of CC16 and its potential as a therapeutic target. The authors also point out the potential major confounding impact of the use of inhaled corticosteroids (ICS) in patients with

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severe asthma. In their study, not surprisingly, high-dose ICS and systemic corticosteroids were significantly associated with severe asthma and low CC16 expression levels in BECs. Critically, adjusting for ICS dose eliminated the association between CC16 concentrations and asthma severity. The authors propose that this may be because of overadjustment. However, it is difficult to untangle the influence of ICS use in this study, which represents a major challenge in translational asthma research in general. Studies of the effect of ICS on BEC CC16 expression in normal individuals and *in vitro* BEC studies would clarify and contextualize the findings of this publication. Finally, the authors present CC16 as a nontraditional Th2 biomarker rather than a negative T2 biomarker. If this is truly the case, then a better understanding of its mechanistic contribution to asthma will be crucial.

Although Li and colleagues from SARP present a promising airway biomarker, there are still important questions that need to be addressed, including identifying the molecular mechanism of CC16 in asthma and the influence of corticosteroids on its utility as a biomarker. However, the approach itself is an important illustration of the path toward refined asthma endotypes. ■

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References

1. Papi A, Brightling C, Pedersen SE, Reddel HK. Asthma. *Lancet* 2018;391: 783–800.
2. Wan XC, Woodruff PG. Biomarkers in severe asthma. *Immunol Allergy Clin North Am* 2016;36:547–557.
3. Denton E, Price DB, Tran TN, Canonica GW, Menzies-Gow A, FitzGerald JM, *et al.* Cluster analysis of inflammatory biomarker expression in the international severe asthma registry. *J Allergy Clin Immunol Pract* 2021; 9:2680–2688.e7.
4. Menzies-Gow A, Corren J, Bourdin A, Chupp G, Israel E, Wechsler ME, *et al.* Tezepelumab in adults and adolescents with severe, uncontrolled asthma. *N Engl J Med* 2021;384:1800–1809.
5. Bernard AM, Roels HA, Buchet JP, Lauwerys RR. Serum clara cell protein: an indicator of bronchial cell dysfunction caused by tobacco smoking. *Environ Res* 1994;66:96–104.
6. Guerra S, Halonen M, Vasquez MM, Spangenberg A, Stern DA, Morgan WJ, *et al.* Relation between circulating CC16 concentrations, lung function, and development of chronic obstructive pulmonary disease across the lifespan: a prospective study. *Lancet Respir Med* 2015;3: 613–620.
7. Li X, Guerra S, Ledford JG, Kraft M, Li H, Hastie AT, *et al.*; NHLBI Severe Asthma Research Program (SARP). Low CC16 mRNA expression levels in bronchial epithelial cells are associated with asthma severity. *Am J Respir Crit Care Med* 2023;207:438–451.
8. Peters MC, McGrath KW, Hawkins GA, Hastie AT, Levy BD, Israel E, *et al.*; National Heart, Lung, and Blood Institute Severe Asthma Research Program. Plasma interleukin-6 concentrations, metabolic dysfunction, and asthma severity: a cross-sectional analysis of two cohorts. *Lancet Respir Med* 2016;4:574–584.
9. Yao Y, Welp T, Liu Q, Niu N, Wang X, Britto CJ, *et al.* Multiparameter single cell profiling of airway inflammatory cells. *Cytometry B Clin Cytom* 2017;92:12–20.

A Few Bad Airways Can Wreak Havoc: Recognizing Asthma as a Local Disorder

Stimulating airway smooth muscle (ASM) in an individual with asthma can cause some airways to hyperconstrict in ways healthy airways would not. Although several reasons could contribute to this, there is unequivocal experimental evidence that the ASM surrounding many asthmatic airways is thicker, and more so with severe asthma (1). Starting with Lambert and colleagues (2) and continued with increasing more morphometrically and three-dimensionally consistent airway trees (3–5), computational models consistently show that if one assumes maximum ASM force generation scales proportionately with its thickness, the thickened ASM found in asthmatic airways can lead to hyperresponsiveness. The above, however, is inadequate to fully understand how lung function degrades during an asthma attack in a specific patient or to guide optimal treatment strategies in a patient-specific fashion. The percentage of ASM thickening is not the same everywhere, either in large versus small airways or across airways of similar original diameter throughout the lung or across subjects with a similar clinical classification of asthma severity (6, 7).

Surely all airways do not constrict identically during an asthma attack; ergo, heterogeneous constriction is a fundamental feature of asthma (8, 9). In fact, this feature is the most important contributor to the degradation of lung function that occurs during an attack (3–5). Models predict that ASM thickening enhances the heterogeneity of the resulting constriction (4). Hence, it would seem useful to identify the precise locations of abnormal ASM thickening. Stated another way, understanding the average constriction across all airways is of limited value when conceptualizing more effective future treatment methods. Rather, it is all about the anatomic origins of heterogeneity. This last statement speaks to the heart of insights from the new study by James and colleagues (pp. 452–460) in this issue of the *Journal* (10).

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