The Intersection between Autoimmunity, Macrophage Dysfunction, Endotype, and Exacerbations in Severe Asthma

Asthma is a clinical syndrome with common features of airway dysfunction and chronic inflammation, but there is considerable disease heterogeneity, which is generally related to the primary inflammatory mechanisms responsible for sustained airway inflammation. Type 2 (T2) inflammation is the best understood mechanism and is typically associated with airway eosinophilia, whereas individuals without biomarkers of T2 inflammation and/or who have neutrophil-predominant or paucigranulocytic inflammation are included under the broad umbrella of non-T2 asthma (1). There is also a subset of individuals with mixed airway eosinophilia and neutrophilia (mixed granulocytic asthma) who exhibit more severe and difficult-to-control disease (2–4). Recent studies have identified associations between autoimmune-related inflammation and uncontrolled disease in these patients, but the specific mechanisms of how autoimmunity contributes to asthma pathogenesis are not well understood.

In this issue of the *Journal*, Son and colleagues (pp. 427–437) enhance our understanding of the role and specific mechanisms of autoimmunity in asthma by characterizing an autoantibody in induced sputum samples obtained from individuals with severe asthma that targets macrophages (5). These investigators have previously identified an autoantibody targeting eosinophils (anti–eosinophil peroxidase IgG) that promotes eosinophil cytolysis and degranulation in individuals with oral corticosteroid–dependent eosinophilic asthma and mixed granulocytic asthma (6). Here, Son and colleagues characterize an autoantibody that targets the macrophage receptor with collagenous structure (MARCO), which they have previously identified in the airways of individuals with severe asthma and recurrent airway infections (7). MARCO is primarily expressed on the cell surface of macrophages in the airways and alveolar spaces, where it serves as a scavenger receptor, acting as a first line of defense in the innate immune response, particularly to bacterial products (8). The authors first measured anti-MARCO IgG levels in induced sputum from a cohort of individuals with severe asthma and detected the autoantibody in 36% of individuals (52 of 143). The autoantibody levels were more prevalent and at higher titers in those classified as mixed granulocytic asthma but were also more frequently detected in individuals with eosinophilic asthma requiring treatment with oral corticosteroids. Individuals with anti-MARCO antibodies were also more likely to have concordant levels of anti–eosinophil peroxidase antibodies, suggesting that broader autoreactivity against multiple antigens occurs concurrently in patients with airway autoimmunity. Multivariate regression analyses identified associations between anti-MARCO IgG and peripheral eosinophilia, lymphopenia, and higher airway eosinophil activity. Anti-MARCO IgG titers were also associated with elevated sputum levels of IL-15, which has been implicated in autoimmunity (9), as well as IL-12 and IL-13, demonstrating the overlap of type 1 and type 2 immune responses that are present in the airways of this patient population.

The authors also examined associations between anti-MARCO IgG and acute exacerbations, finding that anti-MARCO IgG levels were higher in sputum samples collected from individuals at the time of acute exacerbation than in those with controlled disease. Importantly, they found that individuals with high anti-MARCO IgG titers had a shorter time to acute exacerbations with neutrophil-predominant inflammation, and they frequently identified common airway bacterial pathogens in sputum cultures during these events. These findings suggested that anti-MARCO IgG may promote dysfunctional macrophage responses to bacterial pathogens, increasing the risk for infection-related exacerbations in this population. The authors examined the effects of anti-MARCO IgG immunoprecipitated from sputum on monocyte-derived macrophages (MDMs) obtained from healthy individuals, demonstrating reduced granulocyte–macrophage colony–stimulating factor and IL-10 release after stimulation with LPS and IFN-γ and reduced bacterial uptake. Finally, MDMs obtained from three individuals with mixed granulocytic asthma and high sputum anti-MARCO IgG titers demonstrated reduced bacterial uptake after treatment with their autologous sputum autoantibodies. These studies provide a strong biological basis for the associations between infection-related exacerbations in individuals with high anti-MARCO IgG titers.

Autoimmunity has increasingly been implicated in asthma, but our understanding of the mechanisms linking autoantibodies to asthma pathogenesis remains limited. Chronic airway inflammation and cell damage promote release of self-antigens that can trigger local autoantibody production, and numerous autoantibodies have been detected in asthma (10). This study indicates that autoimmunity to lung macrophages may contribute to asthma and infection-related exacerbations. Lung-resident alveolar macrophages are highly phagocytic and thought to tonically suppress inflammation, whereas newly recruited MDMs may augment inflammation (11–14). Recent studies in humans have shown that *MARCO* expression within the lung is variable between macrophage subpopulations, with higher expression in resident macrophages and lower expression in recruited monocyte-like subpopulations (15–17). It is tempting to speculate that anti-MARCO antibodies may preferentially target resident macrophages, predisposing individuals to infection-related exacerbations because of impaired clearance of infection through phagocytosis and dysregulated inflammatory responses. These results set the stage for future experiments that examine whether anti-MARCO autoantibodies differentially regulate these key macrophage subsets and whether this impacts the overall balance of pro- and antiinflammatory responses in the airways.

The findings presented in this article also have significant clinical implications. First, this study indicates that airway autoimmunity may be particularly relevant in patients with refractory airway eosinophilia and mixed granulocytic inflammation, and the authors provide some early insights into clinical parameters that may identify patients with higher likelihood of airway autoimmunity (7). Second, patients with airway autoimmunity may be more likely to develop acute exacerbations with airway neutrophilia, which are more often driven by infectious etiologies that require treatment with antimicrobials rather than escalation of steroids. Last, airway autoimmunity likely represents an inflammatory mechanism that is refractory to escalation of corticosteroids and may be less responsive to currently available biologic therapies used for asthma (7). Given that the overall approach to management of other autoimmune diseases requires further immune suppression, the recognition that this specific set of autoantibodies is involved in the susceptibility to infection suggests that a nuanced approach will be needed as this area evolves.

There are also some limitations of these results to consider, most notably that the authors did not evaluate the role of acute viral infections in this study, which serve as a critical exacerbation trigger in asthma. The role of respiratory viruses in promoting eosinophilic versus neutrophilic exacerbations remains poorly understood, particularly in adult patients with severe asthma (18). Overall, this study provides important insights into the role of autoimmunity in asthma and characterizes a specific pathway through which autoimmunity affects the propensity for neutrophilic asthma exacerbations. Identifying such autoantibodies has the potential to alter asthma management through increased recognition of the potential need for antibiotics to treat exacerbations in selected individuals and/or development of additional pharmacologic strategies that alter autoantibody production. Finally, these results further highlight the complex interplay between T2 and non-T2 mechanisms of airway inflammation, which have overlapping features that can concurrently be present in individuals with severe uncontrolled asthma and may be insufficiently targeted by our current armamentarium of asthma-directed therapies. ∎

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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