What Is "Normal" When Examining Myeloid Cells in Human Airways?

Discovered more than 130 years ago, macrophages have been found in virtually all organs and tissues of the body. Within the lung, macrophages exist in distinct compartments, such as the lung interstitium (peribronchial, perivascular, and perialveolar), and within the airspace (1). Lung macrophages exhibit a variety of functions that depend on their ontogeny and tissue location. Airspace macrophages are sentinel cells located at the interface between the external and internal environments and are critical for tissue homeostasis, protection from pathogens, and clearance of dead cells and debris (2). In addition to these protective roles, airspace macrophages also generate inflammation and can drive tissue injury and repair processes resulting in fibrosis and emphysema (3, 4). The diverse functional roles of airspace macrophages within the human lung is an area of active research. Recent events have demonstrated that some individuals are uniquely susceptible to different injuries or disease states. The question remains as to whether baseline composition and/or function of airspace macrophages are distinct. The definition of "normal" in terms of macrophage subpopulations within the lung needs to be addressed.

To answer these questions, recent studies have focused on characterizing lung macrophage subpopulations and identifying their functions using lineage labeling in rodents and flow cytometry–based studies (5–7). Although these studies have led to understandings on the diversity of macrophages in the lung, they have not untangled the complexity of pulmonary macrophage subsets, particularly in humans, in whom the use of genetic tools is not feasible. Expanded use of single-cell RNA sequencing (scRNAseq), which defines gene expression patterns of individual cells to identify clusters, has overcome some of these prior limitations. In this issue of the *Journal* (pp. 946–956), Mould and colleagues performed scRNAseq on BAL samples from 10 individuals to address the diversity of macrophages in the airspace of normal, healthy adults (8). Consistent with previous studies (9), the majority of the cells found in the BAL were of myeloid origin, with only a small percentage of lymphocytes or epithelial cells. Contrary to prior flow cytometry studies suggesting that airspace macrophages are homogenous, they identified distinct subsets segregated by specific gene expression profiles. The dominant population of cells expressed genes related to normal macrophage homeostatic functions, such as cell adhesion, phagocytosis, and lipid metabolism. They also observed two smaller clusters of macrophages, with one subset expressing specific proinflammatory genes, such as CXCL10, CCL3, and CCL4, and another subset expressing genes associated with metal-binding functions, such as MT1M, MT1A, and MT1H. These data support the conclusion that a diversity of macrophage subsets exist in a normal, healthy human lung.

Beyond the observation of macrophages, they also observed clusters of monocyte-like cells from within the BAL samples (8). Through the use of pseudotime analysis, the investigators determined that some of these monocyte clusters were in a state of transition into alveolar macrophages. Another cluster expressed genes such as MMP14 and SPP1, which were associated with cell–matrix interactions. To further characterize these monocyte-like cells, they correlated their gene expression profiles to a previously published scRNAseq dataset of monocytes isolated from peripheral blood samples of healthy male donors. Although there were similarities noted between the two samples sets, there were also distinct gene expression profiles noted between monocytes isolated from the airspace compared with those isolated from the blood stream. Together, these data suggest a steady state trafficking of monocytes into the lungs even in the absence of detectable inflammation and disease. This is particularly interesting given that prior research in rodents suggests that alveolar macrophages self-renew without contribution from circulating cells in steady state (6), which raises questions regarding differences in macrophage heterogeneity between mice and humans.

Overall, this work examines how "normal" is defined in the lung. Although lineage labeling and other genetic studies in rodent suggest that lungs macrophages are homogeneous, the human airspace macrophage pool is clearly more complex. A likely explanation for this contrast is that rodents are raised and maintained in the largely sterile environment of research animal facilities, with filtered air and limited exposure to respiratory pathogens. Alternatively, humans are regularly exposed to respiratory pathogens, air pollutants (e.g., ozone and particulate matter), and indoor dusts and chemicals. As metals are known products of combustion and associate with particulate matter (10), it is not surprising that there is a subset of airspace macrophages with gene expression associated with metal binding. In addition, recent research suggests that long-term exposure to particulate matter $\leqslant 2.5$ μm in aerodynamic diameter in mice leads to a change in airspace macrophage composition (11). This suggests that these daily interactions with the environment likely alter the "normal" human lung. The questions going forward are how do animal models need to be adjusted to account for this heterogeneity in "normal" humans and whether these individual subsets observed represent transient or permanent subsets of airspace macrophages.

Another important question is the role of sex in regulating gene expression profiles within the lung. Interestingly, though perhaps not surprisingly, the composition of individual macrophage clusters were unchanged between male and female subjects. Although the authors did not observe overall significant differences between these

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subgroups, some genes did exhibit opposing expression patterns between sexes (8). It is not clear whether this could explain some of the differences in sex responses to lung injury (12). Additional caveats need to be clarified, such as normalization for menstrual cycle, which could alter macrophage-specific gene profiles. Also, these studies did not include an inflammatory insult, in which the majority of the sex-dependent effects have been observed (13). These studies provide an important foundation to continue to examine the role of sex hormones in regulating macrophage gene expression within the lung.

Taken together, this study provides a concise, well-annotated database for the characterization of lung airspace macrophages from multiple healthy donors. They observed consistent gene expression profiles at a single-cell level between individuals, defining distinct subsets of airspace macrophages with unique profiles. In addition, they provide evidence that there is a constant stream of monocytes into the lungs even in the noninflammatory state. If true, these studies suggest that the airspace macrophage pool is more complex than previously identified, and this reflects a new "normal" that must be considered in human response to injury and chronic disease. ■

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Mucus Plugs in Medium-sized Airways: A Novel Imaging Biomarker for Phenotyping Chronic Obstructive Pulmonary Disease

Multiple studies have already focused on the phenotyping of patients with chronic obstructive pulmonary disease (COPD) by assessing emphysema and airway disease on chest computed tomography (CT). In 2015, the Fleischner Society proposed to differentiate between emphysema-predominant and airway-predominant imaging subtypes based on the presence of at least 5% pulmonary emphysema (1). Additionally, it defined five different subtypes of emphysema-predominant phenotypes based on a visual assessment of the severity and pattern of emphysema (1). More recently, an ancillary study from the COPDGene (Genetic Epidemiology of Chronic Obstructive Pulmonary Disease) study described 10 nonoverlapping CT imaging subtypes by combining visual and quantitative CT imaging features (2). Although emphysema can be simply quantified by measuring the percentage of lung voxels below −950 Hounsfield units (3), the assessment of airway disease is usually more complex. Bronchial airway disease can be estimated

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