Know Where You Are: Pulmonary Macrophage Locations in the Human Lung

Historically, macrophages have been defined by their ability to clear debris from injured and/or damaged tissues, thus, the term "big eaters." However, "big eaters" fails to encompass their diverse functional repertoire. Data spanning a variety of organ systems support various homeostatic and injury or repair functions for macrophages, which are likely directed by local tissue conditions and signals. In fact, the sheer diversity of functional responses has made it difficult to classify macrophages. One attempt has been to define macrophages by polarized functional outcomes. This led to nomenclature such as "classical versus alternative activation" or "M1 versus M2" (1). Though these functional characterizations may occur in carefully selected *in vitro* conditions, their relevance *in vivo* or in complex biological settings has not been definitively demonstrated. Alternatively, recent flow cytometry–based rodent studies have defined macrophage-based tissue location (i.e., alveolar space = alveolar macrophage [AM] and interstitial space = interstitial macrophage [IM]) (2–5). Simultaneous studies by our group and two other groups extended this flow cytometry approach to human lung tissue (6–8). However, none of these studies definitively confirmed the tissue location of macrophages. Therefore, a detailed histologic analysis defining macrophage locations and quantification in the interstitial or alveolar spaces has been lacking.

In this issue of the *Journal*, Hume and colleagues (pp. 1209–1217) address this gap by clearly defining the tissue location of human macrophages using design-based stereology (9). This methodology quantified AMs and IMs in lung tissue to a level of precision not previously available. Beyond general quantification of AMs and IMs, the authors further segregated macrophages into specific tissue locations. For example, of the airspace-localized macrophages, 95% resided in the alveolar spaces, whereas 5% resided in the airways. IMs were divided into those that populate the alveolar septa with smaller identified groupings around vessels and airways, respectively. Performing simultaneous flow cytometry on the lung tissues, they compared the differences between quantification by stereology with flow cytometry–derived quantification; demonstrating that flow-based methods underestimate IM numbers. Finally, using lung tissue samples from smokers and nonsmokers, and from males and females, they performed an assessment of the impact of these variables on the proportions of macrophages in each compartment. Interestingly, they noted that AM numbers in their cohort were not increased in smokers; rather, there was an increase in IM numbers. Alternatively, they did not note an impact of sex on macrophage numbers either in the healthy controls or the smokers.

This work seeks to address some important controversies in defining pulmonary macrophages. Though several groups have performed histology to define pulmonary macrophage subsets, the spatial resolution of these limited images have not directly clarified specific macrophage tissue locations. This limitation led us, in a prior study, to define IMs as "interstitial-associated macrophages" (7). The present study resolves this controversy and validates prior flow cytometry approaches segregating AMs and IMs. Additionally, the authors address concerns raised regarding the quantification of tissue macrophages using flow cytometry (10). They demonstrate that flow cytometry may inaccurately quantify IM numbers. A potential caveat to this observation is that IM exhibit dendrite-like processes through tissue structures. Individual dendrites, despite Z-stacking, could be counted as individual cells, which could artificially elevate the IM counts. However, this also raises important potential effects of tissue digestion on cell yield, particularly from tissue structures. These effects need to be carefully considered by investigators using tissue digestion to obtain IM for functional studies or when performing single-cell sequencing studies in which digestion is required to obtain single-cell suspensions.

An important implication of this study is the demonstration of pulmonary macrophages in distinct tissue locations in the airspace and lung tissue. Despite the authors' caveat that tissue processing might push airway macrophages into the distal airspace, they clearly distinguish macrophages residing in the airway from those in the alveolar space. Additionally, they define groupings of IMs located in distinct tissue regions, including the alveolar septum, and around the vasculature and the airways. The question not addressed in the study is if there are specific surface markers that define the macrophages in these specific tissue locations. For example, prior work by this group identified three distinct murine IMs that exhibited distinct genetic programs (5). It would interesting know if unique surface markers could be defined for IMs in these specific lung tissue locations and if these macrophages exhibit distinct functions. This would be expected based on their locations where individualized signals are likely provided by those niches. In support of this concept, recent work suggests that macrophages around the interstitium sense hypoxia and regulate vascular remodeling (11). Future work would require a greater understanding of the function of these macrophages in distinct structures and if different regions support specific macrophage functions.

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One of the most provocative observations is that, by stereology measures, smokers do not appear to have an increase in AM numbers. This is in conflict with studies demonstrating an increase in BAL macrophages from smokers (12). Interestingly, prior data support the authors' present observation, suggesting that the extent of the response in BAL was overrepresented by an analysis in lung tissue sections (13). Though not directly explored in this study, a possible explanation for these divergent findings is that AMs from smokers may be easier to lavage during BAL, thereby increasing their measured numbers. Alternatively, the authors identified that IMs were increased in smoker lung tissue, which appeared to be mostly due to an increase in macrophages in the alveolar septum. The role of the IMs in this setting were not clearly defined but these data do suggest the potential importance of defining IM function in exposure conditions like cigarette smoke and, ultimately, in disease states like chronic obstructive pulmonary disease.

In total, this study continues to expand on our understanding of macrophages based on lung tissue location. It offers tantalizing insights into further tissue specification of macrophages and suggests that more work needs to be done, both to identify tools for isolation but also to focus on macrophage functions in these distinct regions. Ultimately, further work in these areas will allow the research community to truly grasp the diverse functional roles of macrophages with a goal of being able to tune these functions to limit tissue damage and/or injury and mitigate chronic lung disease. ■

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A Physiological Point of View on Expiratory (Re)action during Mechanical Ventilation

A commonly held belief about avoiding ventilator-induced lung injury primarily takes into account the inflation half-cycle, whereas deflation is considered to be a passive process about which very little can be done to influence the lung function of patients (1). Is this belief actually correct? We know that patients should be ventilated without harming the lung (so-called protective lung ventilation) (2). This may be achieved by combining low V_T with the correct amount of positive end-expiratory pressure (PEEP) to minimize the mechanical load on the ventilated lung. However, mechanical ventilation is different from the physiological mechanism that mammals use for gas exchange, in which the inspiratory flow is obtained by the negative pressure generated by the inspiratory muscle. Expiration is often believed to be passive and determined by the elastic recoil pressure of the lung, as it is during physiological ventilation. Unfortunately, expiration is not an exclusively passive phenomenon. The diaphragm not only acts as an inspiratory muscle but also exerts a braking action aimed at slowing down the expiratory flow (3). The absence of this brake, as in the case of patients with paralysis, is responsible for much more rapid lung

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