The Transcriptional Signature in Alveolar Macrophages Dictates Acute Respiratory Distress Outcomes

Acute respiratory distress syndrome (ARDS) is a severe inflammatory lung disease with high mortality (1). Unremitting lung inflammation portends a poor prognosis for patients with ARDS (2), but pharmacotherapies designed to suppress inflammation have failed to improve outcomes. Although many cell types are involved in tissue repair, macrophages have been shown to exhibit critical activity at all stages of repair and fibrosis due to their highly flexible programming (3). Interest in the role of alveolar macrophages (AMs) in ARDS was sparked in 1994 by Steinberg and colleagues, who showed a progressive increase in AMs on Days 3, 7, and 14 after ARDS onset in survivors compared with no change or a decrease in AMs in nonsurvivors (4). Since then, there has been an evolving recognition of distinct macrophage phenotypes, resulting in the identification of mechanisms that guide macrophages to take on proinflammatory, profibrotic, proinflammatory, antifibrotic, and proresolving roles. Emerging evidence supports the notion that phenotypically distinct AMs mediate different phases of acute lung inflammation and resolution (5). Building on this, persistent increased proinflammatory expression of the AM surface markers CD11b and myeloid-related proteins 8 and 14 was associated with decreased survival in patients with ARDS (6).

In this issue of the *Journal*, Morrell and colleagues (pp. 732–741) advance our understanding of AM transcriptional activation over the first 8 days of ARDS and its association with death or persistent mechanical ventilation at Day 28 (dead/intubated$_{Day28}$) (7). The authors obtained BAL fluid from patients who had been intubated for ARDS at Days 1, 4, and 8, unless they were deceased, had been extubated, or did not meet safety criteria for bronchoscopy. AMs were isolated by negative immunoselection followed by RNA extraction and microarray hybridization. Expression of PD-L1 (CD274) was upregulated in alive/extubated$_{Day28}$ cells on Day 1. Morrell and colleagues recently found that AMs from individuals who experienced a high number of ventilator-free days had significantly higher PD-L1 gene expression than those obtained from subjects who experienced a low number of ventilator-free days (8). Conversely, in this study, *FKBP51* (FK506 binding protein 5) was upregulated in dead/intubated$_{Day28}$ cells. *FKBP51* is a steroid-responsive gene, and in other conditions, such as eosinophilic esophagitis and asthma, it has been suggested to be glucocorticoid responsive (9, 10). Increased AM transcription of the *FKBP51* gene early suggests a subgroup with a proinflammatory phenotype associated with increased dead/intubated$_{Day28}$ but that may derive greater benefit from glucocorticoid administration.

The authors used a gene set enrichment analysis to identify 50 "hallmark" gene sets defined in the Molecular Signature Database. In accordance with previously observed phases of ARDS, the association of AM transcription with alive/extubated$_{Day28}$ varied with different temporal measurements (Days 1, 4, and 8). A proinflammatory "M1-like" pathway that was enriched at Day 1 was associated with alive/extubated$_{Day28}$ and, conversely, persistent M1 polarization enrichment at Days 4 and 8 was associated with dead/intubated$_{Day28}$. Enrichment of an "M2-like" pathway (which is classically associated with healing) at Days 4 and 8 was associated with 28-day survival. Their analysis identified 32 individual genes that were enriched in alive/extubated$_{Day28}$ cells at Day 1 of ARDS and were enriched in dead/intubated$_{Day28}$ cells at Day 8.

This analysis provides important AM transcriptional signatures that were obtained serially over the first 8 days of ARDS, shows their association with survival and mechanical ventilation at Day 28, and underscores the importance of AMs in injury and resolution. Moreover, it reinforces the need to better phenotype patients with ARDS. Numerous clinical trials have failed, in part, due to a lack of subphenotyping and personalized treatment (11, 12). However, this study has some limitations that merit consideration. The RNA profile was assessed by microarray hybridization, which depends on the transcriptional abundance and may not be as accurate as RNA sequencing (13). Furthermore, although the M1 and M2 pathways are included in the Molecular Signature Database, questions remain as to whether this paradigm truly captures macrophage plasticity and diversity *in vivo* (14). Lastly, macrophage transcriptional profiles require confirmation by protein expression and ultimately cellular function. With emerging technologies such as mass cytometry (CyTOF) and multicolor flow cytometry, AMs can be evaluated for the expression of surface markers and transcription factors, and the production of proinflammatory and reparative cytokine profiles.

Serial changes of AM transcriptional profiles in ARDS and their association with mortality and mechanical ventilation at Day 28 have important implications. This study identifies a cellular etiology for differences observed in ARDS mortality. The authors point out that these temporal changes may be responsible for negative trials of granulocyte–macrophage colony–stimulating factor, which has a proinflammatory effect but may have been administered too late (on Days 3–7) (15), and statins, which have an antiinflammatory effect but were administered too early (within 48 h) after ARDS onset (16). In future studies examining the reprogramming of AMs to enhance their prorepair "M2 polarization" using, e.g., IL-4 complex (17) or MEK1/2 inhibitors (18), it will be important to consider the timing of treatment and ensure that it is not administered too soon after ARDS onset, to increase the likelihood of observing a benefit. This study will strengthen the rationale for further alveolar cellular phenotyping for ARDS, and the development of treatments that can target the repair mechanism and not solely dampen early inflammatory responses. ■

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Sequencing Lung Cancer's Sequence

Non–small cell lung cancers (NSCLCs) are becoming increasingly diagnosed at early stage as the diagnostic modalities for detecting small lung lesions have improved in quality over time and the implementation of screening has increased across the world. Although this is good news for patients, we still face the challenge of understanding whether we can push the envelope further and detect and eradicate tumors before they are evident on diagnostic imaging studies. In breast cancer, recent research from Hosseini and colleagues (1) and Harper and colleagues (2) using murine models and analysis of human blood specimens indicates that circulating tumor cells can disseminate before development of a clinically detectable primary tumor. Presumably, these cells derive from microscopic tumors that are clinically silent because of dominant dormancy pathways and/or because of effective immune response. In this issue of the *Journal*, Kadara and colleagues (pp. 742–750) present a comprehensive deep sequencing analysis of tumor and nonmalignant airway epithelium specimens from 48 patients with cancer to examine the sequence of the sequence of spatial mutations in the lung (3). This work sheds important light on molecular and genetic processes involved in lung carcinogenesis, especially during an early phase of its evolution.

More comprehensive genomic analyses have been conducted for a similar biological context, Barrett's esophagus, which is thought to be a premalignant precursor lesion for esophageal adenocarcinoma. These studies showed that nondysplastic metaplastic Barrett's lesions can harbor mutations commonly observed in esophageal adenocarcinomas; however, phylogenetic analyses of multiple lesions of Barrett's esophagus and esophageal adenocarcinomas revealed distinct genomic alterations patterns suggestive of parallel carcinogenic progression (4).

In the hematopoietic system, comprehensive genomic analyses of blood samples collected from a population without hematopoietic malignancy have revealed clonal mutations in specific genes that are frequently observed at a relatively high prevalence in leukemia (5). Although the vast majority of those cases do not progress to

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