

development requires certain standards for analytical validity (meaning that the biomarker needs to be accurate, reproducible, and reliable), clinical validity (ability to separate groups with distinct clinical/biological outcomes or differences), and clinical utility (the use of the biomarker should improve measurable clinical outcomes) (14). When studying the airway microbiota, we are still frequently faced with analytical validity challenges, in part related to the low biomass and risk for reagent contamination (most important for lower airway samples), as well as the lack of uniformity of sequencing techniques and analytical approaches. Further, unlike gut microbiome studies, airway microbiome studies have been small and frequently limited to few centers, even when noninvasive samples, such as sputum, are used. Thus, for the most part, the clinical validity is limited by the single discovery cohort design (such as the one described in this study) and the lack of validation. And finally, as promising biomarkers arise, we need effective strategies to test whether the use of microbiome data can affect clinical outcomes. Thus, the current study is an important initial step in biomarker discovery. The road ahead will require larger cohorts and different designs so we can have a personalized approach in which noninvasive microbial signatures may have clinical implications for patients with COPD. ■

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Jun-Chieh J. Tsay, M.D.
Leopoldo N. Segal, M.D.
Division of Pulmonary and Critical Care Medicine
New York University School of Medicine
New York, New York

ORCID IDs: 0000-0002-2282-2510 (J.-C.J.T.); 0000-0003-3559-9431 (L.N.S.).

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Building Strong Neighborhoods in the Lung with a Little Help from My Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are multipotent stromal cells that can be isolated from numerous tissues, with the most studied

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sources being the bone marrow, skeletal muscle, amniotic fluid, and adipose tissue (1–3). By definition, MSCs must meet the following requirements: 1) adherence to plastic; 2) trilineage differentiation into adipocytes, chondrocytes, and osteoblasts; and 3) expression of cell-surface mesenchymal markers (CD105, CD90, CD73, CD13, CD166, CD44, and CD29) and a lack of expression of hematopoietic and endothelial surface markers (CD45, CD31, and CD34) (4).

Furthermore, key unique features of MSCs are their ability to repair tissue through paracrine support of injured cells, partially due to their transfer of mitochondria into damaged cells (i.e., alveolar epithelium), and their ability to modulate the immune response

in a paracrine manner through the production of a range of immunomodulators (such as TGF- β [transforming growth factor β], PGE2 [prostaglandin E2], IL-10, nitric oxide, and indoleamine 2,3-dioxygenase) and the inhibition of T-cell proliferation (5). All of these features point toward MSCs as one of the most useful cell sources for clinical application in tissue regeneration and cell therapy, with a low risk compared with embryonic stem cells because of their minimal risk of tumor formation and low immunogenicity (6).

Accordingly, the transfer of MSCs has been proposed as a therapeutic tool for acute and chronic lung injuries, and has been successful in animal models of endotoxin-induced acute respiratory distress syndrome, where MSCs decreased alveolar leakage, suppressed inflammation, and improved survival (7–11). In a murine model of bleomycin-induced lung fibrosis, the systemic administration of MSCs was shown to be effective in preventing the development of lung fibrosis (12–14).

A key feature of adult stem cells in general is that they reside in specific anatomical sites or niches (i.e., bone marrow) that preserve their potential, regulate their proliferation, and inhibit their differentiation, preserving their stemness throughout life (15). The decision to lie dormant, self-renew, or differentiate is a consequence of the diverse cocktail of signals provided by the stem cell niche. Recent studies have proposed that the niche not only affects the homeostatic pool of the stem cells but also profoundly affects the functionality and behavior of the cells (15–17). In this context, the microenvironment in which the MSCs reside acts as a director that modifies their functionality (18). In fact, the environment can drive these cells to the fully functional immunosuppressive MSC phenotype or to a “proinflammatory” (impaired in repair) phenotype (19). Key factors in this phenotype modulation are the extracellular matrix itself, as well as reactive oxygen species (ROS) (oxidative stress). Specifically, increased ROS inhibit MSC proliferation, increase senescence, enhance adipogenesis but reduce osteogenic differentiation, and inhibit MSC immunomodulation.

Previous studies have shown that exposure to disease-associated ROS in atherosclerosis and type 2 diabetes impairs the immunomodulatory capacity of MSCs (20, 21). In this issue of the *Journal*, Islam and colleagues (pp. 1214–1224) contribute to the concept of the microenvironment as a key factor in shaping the function of MSCs in the treatment of lung injuries (22). In their study, they induced three different conditions of lung injury with different microenvironments: 1) intratracheal instillation of hydrochloric acid (HCl), 2) mechanical ventilation, and 3) two-hit injury (combining HCl and mechanical ventilation). In these three different injured conditions there were different responses, ranging from no effect to beneficial or detrimental effects, directly after the administration of MSCs. This shows that the microenvironmental modulation of the MSC phenotype takes place *in vivo*. Specifically, in the case of mechanical injury alone, MSC transfer reduced lung injury and fibrotic changes, whereas in mice that received HCl, with or without ventilation, it resulted in greater fibrotic changes (Ashcroft score) and inhibited reepithelialization. The authors were able to demonstrate that MSCs were protective in the HCl model if they were given 14 days after injury, by removing the proinflammatory microenvironment.

Finally, the authors demonstrated that modulation of the microenvironment can be done concomitantly with MSC transfer,

either by GPx-1 administration (correcting the oxidative stress) (23) or by the MSCs themselves when modified to carry human hepatocyte growth factor or human IL-10.

The observations presented in this study are critical, based on the concept that with any therapy we need to prevent harm to the lung. It is reasonable to conclude that the characterization, identification, and optimization of the lung microenvironment would improve the efficacy of MSCs in the treatment of acute respiratory distress syndrome, as well as many other injuries in which there is a proinflammatory microenvironment with a high level of oxidative stress. Interestingly, the data support the need to develop a “second generation” of MSCs, in which MSCs are modified to specifically enhance their therapeutic effect by overexpressing antiinflammatory molecules (IL-10) and protective molecules (hepatocyte growth factor) or by use of microRNAs to regulate protein expression in specific target cells (24), that can lead to a more “secure” and highly effective MSC-based lung therapy. ■

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Rosa Faner, Ph.D.
Institut d'Investigacions Biomediques August Pi i Sunyer
 Barcelona, Spain

and

Centro de Investigación Biomedica en Red Enfermedades Respiratorias
 Madrid, Spain

Mauricio Rojas, M.D.
The Dorothy P. and Richard P. Simmons Center for Interstitial Lung Diseases
 and
Division of Pulmonary, Allergy and Critical Care Medicine
 University of Pittsburgh Medical Center
 Pittsburgh, Pennsylvania

ORCID IDs: 0000-0002-8159-0115 (R.F.); 0000-0001-7340-4784 (M.R.).

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⊗ Teaching an Old Intensivist Neutrophil Tricks: Using Alveolar Neutrophilia to Diagnose Ventilator-associated Pneumonia

An insidious belief pervades the modern ICU—that in cases of diagnostic uncertainty, broad-spectrum antibiotic therapy is “safest for the patient.” This idea is likely a vestige of that bygone era before multidrug-resistant bacteria, when faith in the everlasting efficacy of antimicrobials was strong, and responsible doctors protected their patients from infection without regard for pretest probability.

Fortunately, this belief has been vigorously challenged. Antibiotic overuse is now known to be a clear contributor to the spread of drug resistance, which the World Health Organization has declared to be one of the greatest current threats to human health. It has further predicted that in the absence of improved stewardship, we will enter a postantibiotic era by 2050, with an associated 10 million deaths due to multidrug-resistant bacteria per year (1).

If this apocalyptic prediction were not deterrence enough, it is also clear that inappropriate antimicrobial therapy poses an immediate threat to the patient (2). Risks include life-threatening drug reactions such as Stevens–Johnson syndrome and anaphylaxis, as well as more common complications such as

drug–drug interactions and nephrotoxicity. A more recently appreciated hazard is the profound disruption of gut microbiota produced by antibiotics, a condition termed dysbiosis. Although the full consequences of dysbiosis have yet to be elucidated, it is clearly linked to *Clostridioides difficile* colitis, a highly prevalent and often deadly infection (3). Altogether, the adverse effects associated with unnecessary antibiotics have been shown to worsen mortality in a number of studies by independent groups (4, 5).

It is therefore imperative for both the community as a whole and the individual patient that we develop highly sensitive diagnostic tools to rule out bacterial infection and enable safe and prompt cessation of antibiotics. Such diagnostics are particularly needed for pneumonia, which is responsible for a substantial proportion of antibiotic misuse and is a well-established driver of resistance (6–8).

In this issue of the *Journal*, Walter and colleagues (pp. 1225–1237) take an important step toward solving this problem in the ICU (9). To do so, they make use of the defining host immune response in bacterial pneumonia, namely, neutrophilic alveolitis. Indeed, most of the clinical manifestations of pneumonia stem from this process, including 1) respiratory symptoms such as cough and purulent sputum; 2) systemic signs such as fever, which results from inflammatory signals derived in part from polymorphonuclear cells; and 3) radiographic infiltrates, which in pneumonia represent pus in the lung. Although relatively nonspecific, the BAL

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