the simple review of the morphology of the spirometry curves could lead to the distinction of new subgroups—another example of the pathology's heterogeneity.

In both health and disease, exercise is a model of integrative physiology and provides a great opportunity to study the combined cardiopulmonary insufficiency. Rocha and colleagues have used this model to start understanding the determinants of exercise limitation resulting from a complex interaction between HF and COPD (4). Further research could bring new insights into these patients' behavior. Moreover, measurement of additional variables could provide newer information but simultaneously reveal new subgroups of patients not imagined today. ■

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Extracellular Vesicle Transfer from Mesenchymal Stromal Cells Modulates Macrophage Function in Acute Lung Injury

Basic Science and Clinical Implications

Once considered to be nothing more than nonspecific debris released from dying cells, extracellular vesicles have attracted growing interest from basic and clinical investigators. A wide variety of cells release extracellular vesicles as a response to pathophysiologic stimuli. Extracellular vesicles include exosomes, microvesicles, and apoptotic bodies. Although apoptotic bodies (>1,000 nm) are products of dying cells, exosomes (20–100 nm) are formed by the fusion of multiple endosomes, including lipids, proteins, and nucleic acids. Microvesicles (100–1,000 nm) are formed by budding off from the plasma membrane and contain cellular fractions that include proteins, lipids, mRNA and microRNA, and mitochondria. Both exosomes and microvesicles

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can interact with other cells via ligand–receptor pathways, and they can be internalized, leading to biologic responses (1).

In this issue of the *Journal*, Morrison and colleagues (pp. 1275–1286) report that conditioned media (CM) of bone marrow-derived mesenchymal stromal cells (MSCs) (MSC-CM) contain extracellular vesicles that alter the function of macrophages so that they acquire the capacity to reduce experimental acute lung injury (2). For the *in vitro* studies, human-derived monocytes were differentiated into alveolar-like macrophages that produced increased quantities of tumor necrosis factor (TNF)-α and IL-8 when exposed to endotoxin or bronchoalveolar lavage from patients with acute respiratory distress syndrome (ARDS). When the macrophages were cocultured with MSC-CM, there was a significant reduction in the secretion of TNF-α; enhancement of their expression of the antiinflammatory M2 marker, CD-206; and an increase in phagocytosis of bacteria. A blocking antibody to CD-44 partially inhibited the beneficial effects of reducing TNF-α secretion and the increased phagocytic activity of the macrophages. To test *in vivo* relevance, the authors isolated alveolar macrophages from C57/BL6 mice and treated them *ex vivo* with MSC-CM; the alveolar macrophages were then adoptively transferred intranasally into mice that had been injured with endotoxin. Compared with untreated macrophages, the MSC-CM–treated alveolar macrophages reduced lung injury, as measured by reduced bronchoalveolar lavage concentrations of protein and inflammatory cells. By flow cytometry, the MSC-CM contained extracellular vesicles, and 25% of the vesicles were positive for mitochondria. In addition, these mitochondria were identified in the mitochondrial network of the monocyte-derived alveolar-like macrophages. Using oligomycin as an ATP synthase inhibitor, the antiinflammatory effect and the up-regulation of phagocytosis in MSC-CM–treated macrophages was blocked, providing evidence for mitochondrial oxidative metabolism in the MSC-mediated extracellular vesicle modulation of macrophage function, primarily from transfer of functional mitochondria from the MSCs to the macrophages.

These studies add to a growing body of evidence that the therapeutic effects of MSCs in preclinical models of acute lung injury are mediated in part by extracellular vesicles that transfer biologically active material to host cells, including monocytes and macrophages. Pioneering work in 2012 by Islam and colleagues (3) established connexin-43–dependent mitochondrial transfer from MSCs to alveolar epithelial cells in endotoxin-injured mice, resulting in restoration of surfactant secretion, normalization of alveolar epithelial cell ATP concentrations, and increased survival. More recently, Phinney and colleagues (4) demonstrated mitochondrial transfer from MSCs to macrophages by extracellular vesicles, resulting in enhanced bioenergetics in the macrophages (4), and Jackson and colleagues (5) reported MSCs induced mitochondrial transport by tunneling nanotubes that enhanced macrophage phagocytosis. Research from the laboratory of Dr. Jae Woo Lee has demonstrated that microvesicles from MSCs restore alveolar fluid clearance in *ex vivo* perfused human lungs (6) and reduce lung injury in mice from endotoxin (7) and from live bacteria (8). Also, there was an equivalent therapeutic effect of the MSC-derived microvesicles compared with MSCs themselves. In addition, studies from Dr. Kourembanas's neonatology research group indicate that density gradient preparations of exosomes from MSCs can reverse bronchopulmonary dysplasia in mice, in part by modulating macrophage function (9, 10). Favorable therapeutic effects of MSC-derived extracellular vesicles have also been reported in preclinical models of acute kidney injury (11), myocardial ischemia (12), and traumatic brain injury (13).

What are some of the basic science implications of these studies of extracellular vesicles generated by MSCs? The preclinical studies of ARDS, bronchopulmonary dysplasia, and nonpulmonary organ injury provide evidence that transfer of extracellular vesicles may mediate most of the therapeutic effects of MSCs. However, independent of extracellular vesicles, several secreted soluble factors are present in MSC-CM, including IL-1 receptor antagonist, tumor necrosis–stimulated gene 6 protein, keratinocyte growth factor, angiopoietin-1, lipoxin A4, and prostaglandin E2, all of which have therapeutic effects experimentally in acute lung injury (14). Some of these beneficial proteins and lipids may be produced by the transfer of mRNA in extracellular vesicles released by MSCs to injured epithelium or to activated macrophages. For example, in one study, the transfer of KGF mRNA in microvesicles may have been the main pathway for an increase in KGF protein (8). As another possibility, transfer of mRNA for COX2 could have been the main pathway for increased PGE2 production and part of the beneficial effect of MSCs in experimental sepsis (15). The current study by Morrison and colleagues (2) provides evidence that transfer of mitochondria itself from MSCs through extracellular vesicles can modulate macrophages from a proinflammatory to an antiinflammatory phenotype that might accelerate the resolution of lung injury, findings that are consistent with findings from other studies (5, 8, 10).

What are the clinical implications of these studies? Do these findings mean that cell-based clinical trials with intact MSCs for treating acute organ injury such as ARDS, infant respiratory distress syndrome, or sepsis are misguided? In one preclinical study, MSCs were more effective than MSC-CM in recovery from ventilator-induced lung injury (16). Also, MSCs have been effective in neonatal models of perinatal lung injury (17). Moreover, it is possible that the direct cell contact from intact MSCs in the acutely injured organ might generate soluble factors that can diffuse through the injured tissues and release exosomes and microvesicles that transfer to injured cells and favorably affect the resolution properties of recruited monocytes and tissue macrophages. Although there are challenges with MSC-based therapies, including variations in production and cryopreservation and determining the optimal source (bone marrow vs. umbilical cord, for example), the safety record with MSCs as a therapeutic has been excellent (14). Nevertheless, the growing evidence for the therapeutic effects of extracellular vesicles from MSCs does raise the possibility that a cell-free therapy consisting of exosomes or microvesicles or MSC-CM might be produced that could be tested in patients with ARDS, respiratory distress syndrome of the premature infant, sepsis, acute kidney injury, or traumatic brain injury. Several steps would be needed for this approach to become a reality, including optimization of purification methods for isolation of the required fractions of extracellular vesicles from MSCs, accompanied by a comprehensive characterization of the RNA, microRNA, lipids, and proteins in exosomes or microvesicles that would satisfy regulatory requirements from the U.S. Food and Drug Administration, along with a significant scale-up to provide sufficient quantities for clinical testing.

In summary, much has been learned about the role of extracellular vesicles in mediating the therapeutic effects of MSCs in clinically relevant experimental studies of acute organ injury, with transfer of mitochondria, mRNA, and soluble factors that can improve the function of injured endothelial and epithelial cells and modulate macrophage function to advance repair. ■

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Severity Scores and Community-acquired Pneumonia
Time to Move Forward

Ever since the success of the pneumonia severity index (PSI) (1), a favorite pastime of community-acquired pneumonia (CAP) researchers has been to sort through their databases and try to prove that one score is better than another at predicting an important outcome, typically mortality. Over the past 20 years since the publication of the PSI, more than a dozen scores have been promulgated, some specific to pneumonia and others more generic across all patients with sepsis. Although the addition of a severity score to clinical assessment has been shown to be associated with better patient outcomes, a clear consensus from the dozens of comparative analyses of different scores or even meta-analyses (2, 3) is hard to find.

In this issue of the *Journal*, Ranzani and colleagues (pp. 1287–1297) compare the performance of the criteria for systemic inflammatory response syndrome (SIRS); quick sepsis organ failure assessment (qSOFA); confusion, respiratory rate, and blood pressure (CRB); modified sepsis organ failure assessment (mSOFA); confusion, urea, respiratory rate, blood pressure, age $>$ 65 years (CURB-65); and PSI in a large retrospective cohort of 6,874 patients with CAP from Spain (4). Not surprisingly, the best predictor of mortality

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