Cell Therapy Trials for Lung Diseases: Progress and Cautions

Idiopathic pulmonary fibrosis (IPF) is a catastrophic disease that in most patients is characterized by a progressive nature that confers upon it a high mortality (1). This is aggravated by lack of understanding of IPF pathogenesis, which undermines the ability to model the disease in animals and impedes development of effective therapies. Consequently, with the exception of oxygen administration and lung transplantation, the latter a limited option that improves survival in some patients, no currently effective treatment options are available (2). Despite this, the IPF community has made significant advances, and the last decade saw its transformation into a worldwide network of community- and academic-based organizations that, in conjunction with sponsorship from the pharmaceutical industry and the National Institutes of Health–sponsored IPF network, resulted in the development of rigorous clinical trials (reviewed in Reference 1). Although these were not successful in demonstrating drug efficacy in IPF treatment, they helped clarify the natural history of the disease and documented differences in disease progression, with some patients experiencing exacerbations and rapid progression, whereas others have a more protracted course (1). Genetic studies clarified the familial nature of IPF in a subset of patients and identified genetic mutations compromising telomerase function, thus linking the disease to bone marrow abnormalities (3). Clinical trials also contributed a unique repository of biological materials for identification and validation of biomarkers to follow disease progression (1). In a short period of time, these research initiatives have altered clinical practice by demonstrating that the use of azathioprine is toxic to patients with IPF, effectively eliminating the use of this drug in IPF, thus affirming the "*primum non nocere*" principle (NCT00650091) (4).

Within this historical and scientific context, the IPF community has followed closely the potential clinical application of stem cells in the treatment of this disease. The goals of stem cell therapies in IPF might include (*1*) stabilization or even reversal of inflammation and fibrosis or (*2*) regeneration of damaged tissue. However, the use of stem cells for lung regenerative purposes remains elusive, as we are just learning the principles that may be involved in embryonic and postnatal stem–progenitor cell differentiation. In contrast, the clinical application of mesenchymal stem cells (MSCs) is not predicated on their ability to "rebuild" the damaged lung. Current theories as to their endogenous biologic function include their role as pericytes that line blood vessels and that respond in a patterned manner to counteract local inflammatory stimuli (5). After isolation from bone marrow, adipose tissue, placenta, or other sources, MSCs can be expanded in culture and subsequently administered by systemic or local routes into injured animals. These exogenously administered MSCs localize to sites of active inflammation, through chemotactic signals still being elucidated, and exert patterned antiinflammatory responses depending on the specific inflammatory environment (5). Further, administration of non–HLA-matched allogeneic MSCs appears both feasible and safe (5–7). As such, MSCs have already been tested in a number of clinical scenarios, although there remain many unanswered questions as to optimal source and processing of the MSCs, dosing, route of administration, potential effects of the MSC vehicle, and other practical considerations.

A growing body of preclinical literature supports the efficacy of MSC administration in a range of experimentally induced lung pathologies, including those used as models of acute lung injury, lung infections, asthma, bronchopulmonary dysplasia, bronchiolitis obliterans, chronic obstructive pulmonary disease (COPD), pulmonary ischemia–reperfusion injury, and pulmonary hypertension as well as in models of septic shock (reviewed in Reference 8). These studies consistently show that MSC administration ameliorates part or all of the model-specific disease endpoints. MSCs are hypothesized to reduce inflammation primarily by release of soluble anti-inflammatory mediators and microvesicles without the need for engraftment or recapitulation of lung morphogenesis. Other mechanisms may be involved, and although significant deficiencies persist regarding our understanding of the disease-specific molecular mechanisms by which MSCs mediate these ameliorating effects, these studies provide a solid rational basis for the clinical application of MSCs in human lung diseases.

With respect to MSC effects on lung fibrosis, a systematic review of the English language literature identifies 12 published manuscripts that have investigated the effects of syngeneic, allogeneic, or xenogeneic MSC administration in mouse, rat, or pig models of bleomycin-induced lung fibrosis, the most commonly used preclinical model (8, 9). Notably, MSC administration by either systemic or intratracheal route during the early stage of the injury ameliorates acute inflammation and is protective against subsequent development of fibrotic changes. These effects occur in the absence of what appears to be any substantive engraftment of the MSCs in the lung, and limited available data so far implicate release of soluble antiinflammatory mediators as contributing to the MSC effects (8, 9). Importantly, administration of MSCs at time intervals longer than 7 days after bleomycin administration had no effect on established fibrotic changes in either mouse or pig lungs (10, 11). Further, in studies in radiation-induced lung fibrosis in rodents where fibrosis is established prior to institution of therapy, MSCs acquired a myofibroblast phenotype and contributed to fibrotic changes (12, 13). Collectively, despite the well-recognized limitations of the currently available animal models of lung fibrosis (14), these preclinical data support a beneficial effect of MSC administration in fibrotic lung diseases, but only if they are administered early in the disease course, during active inflammation and before significant fibrotic changes.

Clinical studies addressing the value of MSCs in human lung diseases have been conducted in a parallel track in which the safety and potential efficacy of MSCs in lung diseases are studied in anticipation of clarification of the molecular mechanisms responsible for MSC effects. These studies are informed by data obtained during the development of clinical trials of MSCs in different organs such as the heart. For example, as part of the surveillance of untoward effects, Hare and colleagues followed the lung function (FEV_1 and FVC) of subjects enrolled in a double-blind, placebo-controlled, dose-ranging safety trial of intravenous allogeneic human bone marrow–derived MSCs (hMSCs) (Prochymal; Osiris Therapeutics, Columbia, MD) in reperfused patients with acute myocardial infarction (MI) (n = 53) and observed that compared with patients receiving placebo, those

Am J Respir Crit Care Med Vol 188, Iss. 2, pp 123–130, Jul 15, 2013
Internet address: www.atsjournals.org

treated with hMSCs showed a greater increase in predicted FEV_1 and FVC relative to baseline, from 3 days through 6 months after infusion (15). On the basis of these data, Osiris Therapeutics sponsored the largest trial conducted to date inhuman lung diseases to examine, in a multicenter, double-blinded, placebo (vehicle)-controlled, randomized trial the safety and potential efficacy of non–HLA-matched allogeneic bone marrow–derived hMSCs in subjects with moderate-to-severe COPD (16, ClinicalTrials.gov identifier: NCT00683722). Sixty-two patients were recruited at six sites to receive four monthly infusions (100×10^6 cells/infusion) of hMSCs and to undergo periodic evaluations of lung function, quality of life, 6-minute walk, and assessment of systemic inflammation for 2 years. Although this study was not statistically designed to test endpoints of efficacy, it found no significant toxicity, including no clinical signs or symptoms of significant pulmonary emboli during hMSC infusion, or of COPD exacerbations or mortality over the 2-year follow-up period. In addition, an early significant decrease in levels of circulating C-reactive protein was observed in hMSC-treated patients who had elevated CRP levels at study entry, suggesting an antiinflammatory effect of the MSCs.

A Pulmonary Perspective in this issue of the *Journal* (pp. 133–140) (17) and a related press release (18) by Toonkel and colleagues outline the rationale and describe plans for an investigation of MSC administration to patients with IPF. This trial will complement other ongoing safety investigations using MSCs of adipose or placental origin in patients with IPF taking place in Greece and Australia (19, Clinicaltrials.gov NCT01385644). Given our interest in pursuing clinical trials of MSCs in lung diseases, a carefully designed Food and Drug Administration–compliant safety trial of MSCs in IPF is welcome. The role of the Food and Drug Administration is critical to promote ethical, safe, and transparent conduct of the trial and thus to provide an alternative to some patients with IPF who would otherwise resort to medical tourism where the scientific and ethical standards are unknown. Although Toonkel and colleagues make a compelling argument for initiation of IPF trials using MSCs, there are some unanswered questions that are worthy of discussion at this time.

These investigators indicate that they are capitalizing on the available preclinical data using mouse models of bleomycin-induced lung fibrosis and also the experience of clinical trials of MSCs in heart diseases to delineate the clinical trial on IPF. The clinical findings of Hare and colleagues (15) on FEV_1 and FVC in hMSC-treated patients with acute MI were used by Osiris in their design of the COPD trial, and this trial clarified the possibility of administering repeated doses of large numbers of MSCs to an older sicker cohort of patients with severe lung disease (16). However, the recently published POSEIDON trial of autologous versus allogeneic MSC administration in patients with decompensated heart failure by the same group indicates differences in effects of autologous versus allogeneic MSCs, and that low rather than high MSC doses appear to have a more beneficial effect on cardiac function (20). Therefore, data gathered from multiple trials by the same group that will be conducting the IPF safety trial introduce further questions that need to be reconciled about the source and dosing of MSCs and their relative effects on cardiac versus pulmonary endpoints. In particular, questions remain about autologous versus allogeneic bone marrow MSC use, as IPF has been linked to genetic predispositions mediating abnormities in bone marrow function, suggesting potential abnormalities in MSCs obtained from patients with IPF, although a small study suggests that MSCs isolated from patients with IPF appear to have the same telomere lengths as age-matched controls (3, 21).

Further, acknowledging the inadequacy of currently available models of lung fibrosis, there are no current preclinical data to support an effect of MSCs to reverse or slow progression of already established lung fibrosis. The question of potential MSC efficacy thus merits further consideration as the authors are unclear about specifically what inclusion and exclusion criteria will be employed in the trial. Is there a way to include patients earlier in the course of IPF to increase the probability of benefit and lower risk, a difficult prospect, as acknowledged by the authors? Will inclusion criteria be defined by natural history of disease (which has no clear pathogenesis and is variable in its progression), disease biology (fibroblast proliferation with concomitant paradoxical apoptosis of alveolar epithelium), or biologic properties of the MSCs as they "go to the lung and stimulate other cells into making new tissue"? It is entirely possible that in established lung fibrosis, MSCs could lead to the aggravation of the fibrotic process, and there are no currently available data that contradict this potential risk. Addressing these questions is fundamental because it will not only determine the mechanism of action but also the potential toxicity of MSCs in patients with IPF. In this regard, the IPF community could benefit from upcoming clinical trials of MSCs in acute lung injury to consider the use of MSCs in the treatment of highly lethal acute IPF exacerbations. Clinical investigations of MSCs in IPF and other lung diseases are a welcome advance, but we must balance the potential risks while clarifying the cellular and molecular mechanisms associated with MSC use in specific lung diseases. Patients should be encouraged to participate, and the medical community should be well informed about the indications and potential complications associated with their clinical application.

Author disclosures are available with the text of this article at www.atsjournals.org.

Daniel J. Weiss, M.D., Ph.D.
Department of Medicine
University of Vermont College of Medicine
Burlington, Vermont

Luis A. Ortiz, M.D.
Division of Occupational and Environmental Health
Graduate School of Public Health at the University of Pittsburgh
Pittsburgh, Pennsylvania

References

1. King TE Jr, Pardo A, Selman M. Idiopathic pulmonary fibrosis. *Lancet* 2011;378:1949–1961.
2. American Thoracic Society (ATS); European Respiratory Society (ERS). Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement.*Am J Respir Crit Care Med* 2000;161:646–664.
3. Alder JK, Chen JJ, Lancaster L, Danoff S, Su SC, Cogan JD, Vulto I, Xie M, Qi X, Tuder RM, *et al.* Short telomeres are a risk factor for idiopathic pulmonary fibrosis. *Proc Natl Acad Sci USA* 2008;105:13051–13056.
4. Raghu G, Anstrom KJ, King TE Jr, Lasky JA, Martinez FJ; Idiopathic Pulmonary Fibrosis Clinical Research Network. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. *N Engl J Med* 2012;366:1968–1977.
5. Keating A. Mesenchymal stromal cells: new directions. *Cell Stem Cell* 2012;10:709–716.
6. Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, Granton J, Stewart DJ; Canadian Critical Care Trials Group. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PLoS ONE* 2012;7:e47559.
7. Von Bahr L, Batsis I, Moll G, Szako A, Sundberg B, Uzunel M, Ringden O, LeBlanc K. Analysis of tissues following mesenchymal stromal cell therapy in humans indicates limited long-term engraftment and no ectopic tissue formation. *Stem Cells* 2012;30:1575–1578.
8. Weiss DJ, Bertoncello I, Borok Z, Kim C, Panoskaltsis-Mortari A, Reynolds S, Rojas M, Stripp B, Warburton D, Prockop DJ. Stem cells and cell therapies in lung biology and lung diseases. *Proc Am Thorac Soc* 2011;8:223–272.
9. McNulty K, Janes SM. Stem cells and pulmonary fibrosis: cause or cure? *Proc Am Thorac Soc* 2012;9:164–171.
10. Ortiz LA, Gambelli F, McBride C, Gaupp D, Baddoo M, Kaminski N, Phinney DG. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc Natl Acad Sci USA* 2003;100:8407–8411.

11. Cabral RM, Branco E, Rizzo Mdos S, Ferreira GJ, Gregores GB, Samoto VY, Stopiglia AJ, Maiorka PC, Fioretto ET, Capelozzi VL, *et al.* Cell therapy for fibrotic interstitial pulmonary disease: experimental study. *Microsc Res Tech* 2011;74:957–962.
12. Epperly MW, Guo H, Gretton JE, Greenberger JS. Bone marrow origin of myofibroblasts in irradiation pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2003;29:213–224.
13. Yan X, Liu Y, Han Q, Jia M, Liao L, Qi M, Zhao RC. Injured microenvironment directly guides the differentiation of engrafted flk-1 mesenchymal stem cell in lung. *Exp Hematol* 2007;35:1466–1475.
14. Matute-Bello G, Downey G, Moore BB, Groshong SD, Matthay MA, Slutsky AS, Kuebler WM; Acute Lung Injury in Animals Study Group. An official American Thoracic Society workshop report: features and measurements of experimental acute lung injury in animals. *Am J Respir Cell Mol Biol* 2011;44:725–738.
15. Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, Gerstenblith G, DeMaria AN, Denktas AE, Gammon RS, *et al*. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (Prochymal) after acute myocardial infarction. *J Am Coll Cardiol* 2009;54:2277–2286.
16. Weiss DJ, Casaburi R, Flannery R, LeRoux-Williams M, Tashkin DP. A placebo-controlled randomized trial of mesenchymal stem cells in chronic obstructive pulmonary disease. *Chest* 143:1590–1598.
17. Toonkel RL, Hare JM, Matthay MA, Glassberg MK. Mesenchymal stem cells and idiopathic pulmonary fibrosis: potential for clinical testing. *Am J Respir Crit Care Med* 2013;188:133–140.
18. Cardiac and pulmonary researchers team up to test stem cell therapy for fatal lung disease. *Health Canal* 2012 Sep 10 [accessed 2013 Feb 20]. Available from: http://www.healthcanal.com/lungs-breathing/32814-Cardiac-and-Pulmonary-Researchers-Team-Test-Stem-Cell-Therapy-for-Fatal-Lung-Disease.html
19. Tzouvelekis A, Koliakos G, Ntolios P, Baira I, Bouros E, Oikonomou A, Zissimopoulos A, Kolios G, Kakagia D, Paspaliaris V, *et al*. Stem cell therapy for idiopathic pulmonary fibrosis: a protocol proposal. *J Transl Med* 2011;9:182.
20. Hare JM, Fishman JE, Gerstenblith G, DiFede Velazquez DL, Zambrano JP, Suncion VY, Tracy M, Ghersin E, Johnston PV, Brinker JA, *et al.* Comparison of allogeneic vs autologous bone marrow–derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. *JAMA* 2012;308:2369–2379.
21. Antoniou KM, Margaritopoulos GA, Proklou A, Karagiannis K, Lasithiotaki I, Soufla G, Kastrinaki MC, Spandidos DA, Papadaki HA, Siafakas NM. Investigation of telomerase/telomeres system in bone marrow mesenchymal stem cells derived from IPF and RA-UIP. *J Inflamm* 2012;9:27.

DOI: 10.1164/rccm.201302-0351ED

Triggering Receptor Expressed on Myeloid Cells-2: A New Ally against Sepsis

The importance of sepsis need not be stated for readers of the *Journal*. Although much effort has been made over many years to understand the immunology of sepsis, our understanding of the mechanisms and intermediates that lead to, maintain, and resolve a septic state is still incomplete, and there are thus insufficient opportunities for effective therapeutic intervention. Now an article by Chen and colleagues (pp. 201–212) in this issue of the *Journal* (1) introduces triggering receptor expressed on myeloid cells-2 (TREM-2) as a protective factor during polymicrobial sepsis. TREM-2 has recently generated much interest in the field of Alzheimer's disease, with the discovery of rare missense mutations that significantly increase susceptibility (2, 3). However, the present study returns to TREM-2's roots as an immune signaling molecule. As its name suggests, TREM-2 was originally identified as a receptor on the surface of dendritic cells that signals through the adaptor protein DAP12 (DNAX-activating protein of 12 kD) to promote dendritic cell maturation (4). Subsequent research has identified three related but distinct biological roles for TREM-2: as a phagocytic and immune modulatory receptor on macrophages, as a neuroprotective factor expressed on microglia (reviewed in Reference 5), and as a regulator of osteoclast differentiation (6). Myeloid cell TREM-2 has been found to be induced during inflammation (7), to secrete antiinflammatory cytokines and tame TLR signaling (8–10), and to act as a direct phagocytic receptor for bacteria (11). Thus, TREM-2 may also have a role to play in sepsis.

Chen and colleagues start with the observations that *TREM2* mRNA expression is markedly increased in the abdominal ascites of some patients with sepsis. Although such data indicate a role for TREM-2 during sepsis, they do not show what that role may be, nor how important it is to the outcome, nor even whether it is protective or maladaptive. To answer these questions, Chen and colleagues turned to a mouse model. Experimental ligation and puncture of the cecum (CLP) causes enteric microbes to leak into the abdominal cavity, and so provides a widely used model for polymicrobial sepsis. Chen and colleagues show that both *Trem2* transcript and TREM-2 protein expression are induced in multiple tissues by CLP, peaking about 72 hours after surgery. This is not just a result of cell recruitment, because peritoneal macrophages isolated from septic mice show a marked increase in TREM-2 staining compared with normal controls. They then address the issue of how this elevated TREM-2 influences the outcome of sepsis. A blocking protein impairs bacterial clearance, and injection of bone marrow–derived macrophages constitutively overexpressing TREM-2 improves both survival and bacterial clearance. Thus, TREM-2 is protective in this model of sepsis.

The next important question is, how does TREM-2 exert its effect? There is robust evidence to support a model in which TREM-2 activity exerts an antiinflammatory influence (9, 10), which suggests that the survival benefit of TREM-2 expression may be, at least in part, due to reduced immunopathology. *In vitro* data initially support this model: Chen and colleagues observe increased IL-10 and reduced proinflammatory cytokine production in their TREM-2 transgenic macrophages. However, they did not observe these changes in tissues of septic mice injected with TREM-2 transgenic macrophages, despite reduced bacterial burden. Instead, they present evidence that TREM-2 promotes AKT-dependent phagocytosis and killing of bacteria by TREM-2–expressing macrophages themselves (*see* Figure 1). This is reminiscent of an ongoing debate in the neurodegeneration field, where the importance *in vivo* of TREM-2–mediated phagocytosis of apoptotic neurons and proinflammatory debris and TREM-2–dependent antiinflammatory cytokine section by microglia is unclear (5). Interestingly, a very recent report (12) did observe increased proinflammatory cytokine and reduced Th2 cytokine production after small interfering RNA knockdown of *Trem2* in the corneas of mice infected with *Pseudomonas aeruginosa.* This was associated with impaired bacterial clearance, which, in the absence of phagocytosis data, the authors attributed to the altered Th1/Th2 balance. Thus, it will be important for future studies to distinguish between the antiinflammatory and phagocytic activities of TREM-2 in a given context.