Therapeutic Potential of Mesenchymal Stem Cells for Severe Acute Lung Injury

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Preclinical studies indicate that allogeneic human mesenchymal stem cells (MSC) may be useful for the treatment of several clinical disorders, including sepsis, acute renal failure, acute myocardial infarction, and more recently, acute lung injury (ALI). This article provides a brief review of the biologic qualities of MSC that make them suitable for the treatment of human diseases, as well as the experimental data that provide support for their potential efficacy for critically ill patients with acute respiratory failure from ALI. The article then discusses which patients with ALI might be the best candidates for cell-based therapy and provides a template for the regulatory and practical steps that will be required to test allogeneic human MSC in patients with severe ALI. There is a dual focus on how to design trials for testing both safety and efficacy.
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Abbreviations: ALI = acute lung injury; IL = interleukin; KGF = keratinocyte growth factor; MSC = mesenchymal stem cells; NHBLI = National Heart, Blood, and Lung Institute; PGE$_2$ = prostaglandin E$_2$

E xperimental studies have provided evidence indicating that mesenchymal stem cells (MSC) may be useful for the treatment of a variety of clinical disorders, including sepsis, acute renal failure, acute myocardial infarction, and more recently, acute lung injury (ALI). Based on these preclinical studies, MSC reduce the severity of organ injury as well as enhance recovery.

The objectives of this article are the following: (1) to discuss briefly the general properties of MSC, (2) to review key experimental studies that support the potential value of MSC for treatment of clinical ALI, (3) to consider which patients with ALI might be the best candidates for cell-based therapy, and (4) to outline the steps that will be required to test allogeneic human MSC for patients with ALI.

General Properties of Mesenchymal Stem Cells

MSC are multipotent adult stem cells that were first isolated from the bone marrow and have the capacity to differentiate into bone, cartilage, muscle, fat, and fibroblasts. MSC do not possess the plasticity of embryonic stem cells, but they offer practical advantages because of their ease of isolation and propagation and also because their use does not generate the ethical issues that are often raised by the use of embryonic stem cells.[1] MSC were originally characterized in 1968 by Friedenstein and colleagues,[2] who reported that bone marrow stromal cells were adherent, fibroblastic in appearance, and clonogenic. Subsequent work indicated that adult MSC can be isolated from several human tissues, including bone

marrow, placenta, adipose tissue, and human cord blood. Because there are no MSC-specific cell surface markers, the International Society of Cellular Therapy defined MSC by the following three criteria in 2006: (1) MSC should be adherent to plastic under standard tissue culture conditions; (2) MSC should express cell surface markers, including CD105, CD90, and CD73, and should be negative for other surface markers, including CD45, CD34, CD14, and CD11b; and (3) MSC should have the capacity to differentiate into mesenchymal lineages, including osteoblasts, chondroblasts, and adipocytes, under appropriate in vitro conditions.[3]

Several investigators have reported that MSC have a remarkable ability to modulate the immune system, including the function and response of dendritic cells, T and B cells, and neutrophils, in part through the release of proinflammatory and antiinflammatory cytokines and lipid mediators, such as prostaglandin E_2 (PGE_2).[1,4] MSC also produce angiopoietin-1, a molecule that improves endothelial barrier properties. Furthermore, MSC secrete growth factors that have cytoprotective and repair properties, including vascular endothelial growth factor, keratinocyte growth factor (KGF), and hepatocyte growth factor. Therapeutic delivery of MSC into the acutely injured lung can be accomplished via the airspaces or the circulation and results in interaction of the MSC with both resident and circulating cells, thereby reducing injury and enhancing repair by several mechanisms (Fig 1).

From an immunologic perspective, allogeneic MSC are usually well tolerated by the host and have a low immunogenicity pattern because of constitutive low expression of MHC I and II proteins, and, in general, the lack of T-cell costimulatory molecules, such as CD80 and CD86.[5] The safety record for MSC has been favorable so far in clinical trials. No serious infusional or delayed toxicities have been associated with either autologous or allogeneic MSC in adult and pediatric recipients of allogeneic hematopoietic transplantation, the patient population most studied to date.[6] Osiris Therapeutics, Inc. has sponsored clinical trials of allogeneic unrelated donor MSC (Prochymal) that have included > 2,000 patients with a variety of disease conditions.[7] In a randomized, placebo-controlled, dose-escalation safety study of Prochymal in patients with acute myocardial infarction, adverse events were comparable for MSC- and placebo-treated groups, and no adverse events were attributable to MSC administration.[8] In addition, Osiris released some details of their 6-month interim data report on the use of IV MSC in four separate IV infusions (dose not specified) for moderate to severe chronic obstructive lung disease in 62 patients. There were no safety issues. There was a significant decrease in plasma levels of C-reactive protein in the MSC-treated patients indicating biologic activity of the treatment. There was no significant change in pulmonary function or exercise capacity at 6 months; these outcome variables will be evaluated for 2 years.[7]

Preclinical Studies

Several experimental studies have shown the possible value of allogeneic MSC for a variety of clinical disorders, including sepsis, hepatic failure, acute renal failure, and myocardial infarction.[1] In acute renal failure in mice, IV MSC therapy hastened renal tubular epithelial recovery.[9] IV MSC markedly improved mortality from peritoneal sepsis in mice, an effect that was explained by the release of PGE_2 from the MSC, which reprogrammed alveolar macrophages to increase production of interleukin (IL)-10.[10]

In 2003, Ortiz et al[11] reported that MSC therapy could reduce the degree of fibrosis in bleomycin-induced lung injury in mice, and that the effect did not primarily depend on engraftment. Subsequent work
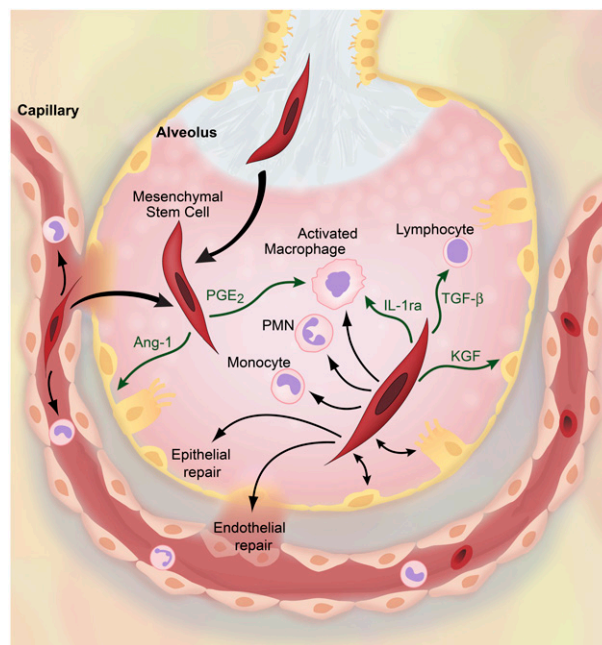

Figure 1. Therapeutic potential of mesenchymal stem cells and their paracrine factors in acute lung injury. This figure shows an injured alveolus with protein-rich edema fluid with an influx of inflammatory cells secondary to both endothelial and epithelial injury. As shown in the diagram, mesenchymal stem cells can be delivered via the air spaces or via the circulation. Some of the potential repair pathways are illustrated as the mesenchymal stem cells interact with injured resident alveolar epithelial or lung endothelial cells or immunomodulate the responses of monocytes, PMN, activated macrophages, and lymphocytes, with the release of several secreted products, including Ang-1, PGE_2, IL-1ra, TGF-β, and KGF. Several other paracrine factors may be important in reducing lung injury and enhancing repair. Ang-1 = angiotensin-1; IL-1ra = interleukin-1 receptor antagonist; KGF = keratinocyte growth factor; PGE_2 = prostaglandin E_2; PMN = polymorphonuclear leukocytes; TGF-β = transforming growth factor-β.

from the same group demonstrated that the release of the IL-1 receptor antagonist by MSC was responsible for this protective effect.[12] Another group reported a survival advantage when MSC were given in bone marrow-suppressed mice.[13] In another study in mice, when endotoxin was administered intraperitoneally (1 mg/kg), MSC prevented endotoxin-induced lung edema and inflammation and also reduced the concentration of proinflammatory cytokines in the plasma.[14]

Our research group studied the effect of MSC on lung injury in nonimmunosuppressed C57BL/6 mice.[15] ALI was induced with a high dose of intraalveolar endotoxin (5 mg/kg). Four hours later, MSC were given by the intratracheal route. Survival was significantly improved with MSC therapy compared with saline controls, as well as controls treated with fibroblasts or apoptotic MSC (Fig 2). MSC reduced the quantity of pulmonary edema and the degree of histologic lung injury. There was also a significant decrease in proinflammatory cytokines and an increase in antiinflammatory cytokines, including IL-10 and IL-13. In additional studies, MSC also reduced mortality in live *Escherichia coli*-induced lung injury in mice, and the number of bacteria recovered from the lung was less with MSC therapy than with saline or fibroblast controls.[15]

In more recent work, we adapted our ex vivo perfused human lung preparation for studies of ALI to test the effects of MSC therapy.[16] In this study, the right middle lobe was injured with intraalveolar endotoxin (6 mg), resulting in a sharp increase in lung endothelial and epithelial permeability to protein leading to pulmonary edema and a loss of the normal capacity of the alveolar epithelium to remove alveolar fluid.[17] When the lung was treated 1 h after instilla-tion of the endotoxin with intrabronchial allogeneic MSC, lung vascular permeability and extravascular lung water returned to normal levels (Fig 3), and the rate of alveolar fluid clearance increased to a normal level (Fig 4A). Control studies with human fibroblasts showed no effect. Interestingly, the beneficial effect of the MSC was reproduced by administering intra-bronchially the culture medium from the MSC. Because we found that the MSC produced a substantial quantity of KGF, we tested the effect of inhibiting KGF release with siRNA knockdown. Approximately 80% of the beneficial effect could be attributed to KGF (Fig 4B). KGF appeared to work by multiple mechanisms, including restoration of lung endothelial and epithelial permeability and an improved capacity of the alveolar epithelium to remove alveolar edema fluid. There are limitations to these ex vivo perfused human lung studies. The experiments were short-term (4 h), and the human lung preparation does not include immune organs, such as the spleen, liver, or systemic lymph nodes. Thus, although a benefit of MSC or conditioned medium could be attributed primarily to the release of a growth factor (KGF), further studies will be needed to determine whether other factors are important in the resolution of lung injury, including antiinflammatory cytokines, other growth factors, and lipid mediators.

Two other recent studies in neonatal experimental models have provided further support for the ability of MSC or the culture medium from MSC to reverse ALI in perinatal hyperoxic models of lung injury. The mechanisms for the benefit were not identified in these studies, but there is clear evidence that paracrine factors released by the MSC play a critical beneficial role.[18-20] One recent study from our group showed that angiopoietin-1 released from hMSC can restore alveolar epithelial permeability to normal in cultured human type II cells.[21] In addition to the studies discussed above, there are several other recent studies that have established the antiinflammatory and barrier protective effects of MSC in the lung, including one in peritoneal sepsis in mice.[22-27]

TESTING OF MSC THERAPY FOR CLINICAL ACUTE LUNG INJURY

To date, there are > 100 MSC clinical trials registered on the clinicaltrials.gov database. These trials encompass a wide variety of human diseases, including congestive heart failure, renal failure complicating coronary bypass grafting, multiple sclerosis, aseptic necrosis of the hip, graft vs host disease, and inflammatory bowel disease. Most of these trials are phase I or phase I/II trials, and limited information is currently available. We are not aware of serious safety concerns arising from these trials. Although the
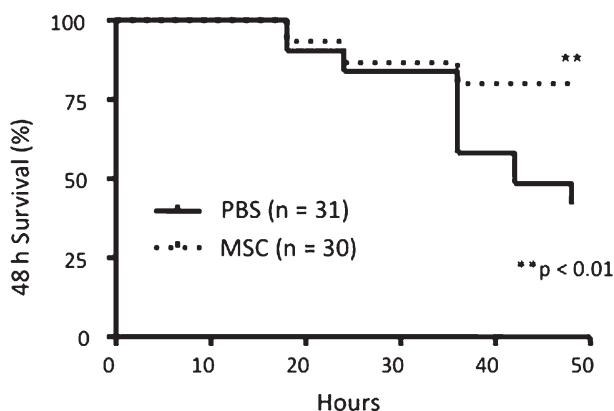
FIGURE 2. Intratracheal treatment with mouse MSC improved survival at 48 h in an endotoxin model of acute lung injury in mice. MSC or PBS was administered intratracheally (IT) 4 h after IT instillation of endotoxin (5 mg/kg). Forty-eight hour survival was 80% in MSC group vs 42% in PBS group (n = 30 for MSC group, n = 31 for PBS group, **P<.01 using a log-rank test). MSC = mesenchymal stem cells; PBS = phosphate-buffered saline. (Reprinted with permission from Gupta et al.[15])
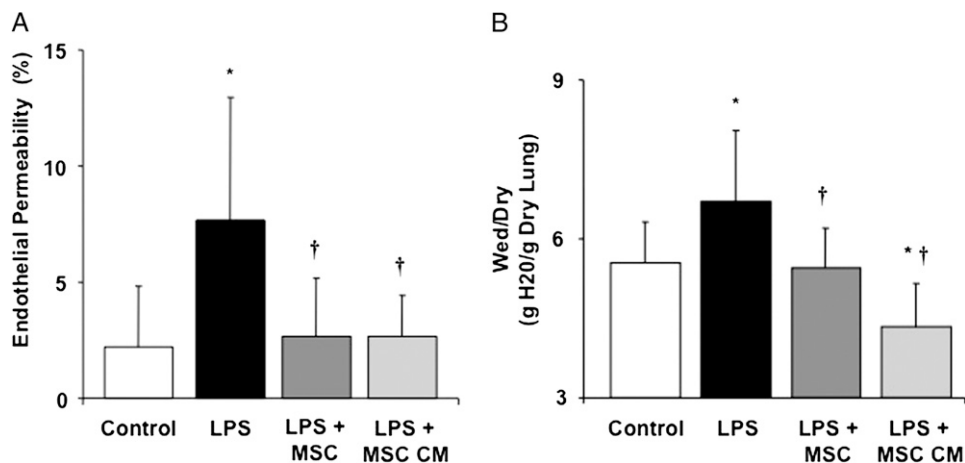
FIGURE 3. Allogeneic human MSC or its conditioned medium restored lung endothelial permeability to protein and wet/dry ratio to normal levels in the ex vivo perfused human lung following injury with *Escherichia coli* endotoxin. Instillation of MSC or its CM into the *E coli* endotoxin-injured (0.1 mg/kg) right middle lobe 1 h following injury restored (*A*) lung endothelial permeability to protein and (*B*) wet/dry ratio to control values. Data are expressed as mean % endothelial permeability or wet/dry ratio ± SD, n = 4 to 5 lungs, *$P < .0001$ vs control lobe, †$P < .0011$ vs LPS–injured (0.1 mg/kg) lobe for lung endothelial permeability, and *$P < .0014$ vs control lobe, †$P < .005$ vs LPS–injured (0.1 mg/kg) lobe for the wet/dry ratio by analysis of variance (ANOVA) (Bonferroni). CM = conditioned medium; LPS = lipopolysaccharide. See Figure 2 legend for expansion of other abbreviation. (Reprinted from Lee et al.[17])

experimental data cited above suggest a potential benefit of MSC cell-based therapy for ALI, considerable work will be needed in clinical trials to evaluate the mechanisms and pathways of benefit and to test both safety and efficacy. Some of these issues for clinical development of MSC in ALI are addressed following.

Patient Selection

No trials have been done with cell-based therapy in critically ill patients with ALI. However, a double-blind, placebo-controlled dose-escalation trial of MSC (IV dosing of 0.5, 1.6, and 5 million cells/kg) in 53 patients treated after acute myocardial infarction has demonstrated the safety of this approach.[8]

Patients who have been enrolled in the National Heart, Lung, and Blood Institute (NHLBI) ARDS Network trials have been identified primarily on the basis of the American European consensus definition of ALI,[28] namely the acute onset of bilateral pulmonary infiltrates and a Pao_2/Fio_2 ratio < 300 without clinical evidence of left atrial hypertension. The mortality of these patients has declined from 40% in 2000, to 25% in the fluid conservative trial in 2006,[29] and most recently to approximately 21% in recent ARDS Network trials,[30] even though the mean Acute Physiology and Chronic Health Evaluation III score has increased over the same time period.[30] Several aspects of improved supportive care have improved clinical outcomes in ALI, including lung-protective ventilation, a fluid-conservative strategy after patients have resolved their shock, and intensive care protocols to prevent nosocomial infections and thromboembolic
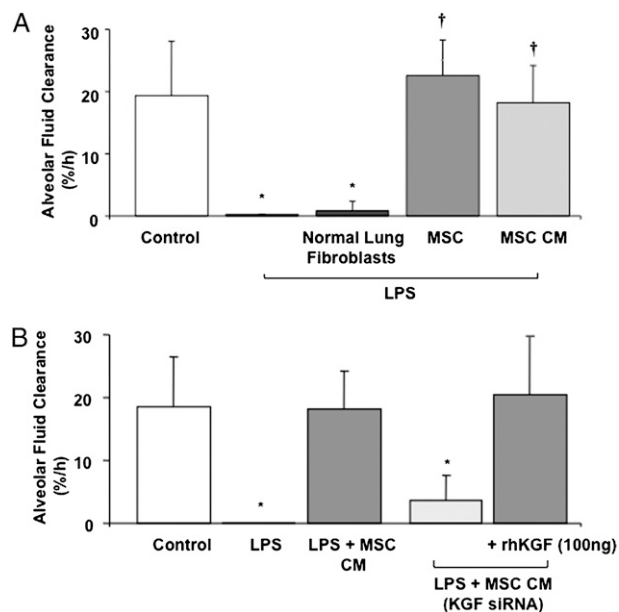

FIGURE 4. (*A*) Allogeneic human MSC or its CM restored alveolar fluid clearance to a normal level in the ex vivo perfused human lung injured with *Escherichia coli* endotoxin. MSC or its CM restored the decrease in alveolar fluid clearance in the lung lobe injured by *E coli* endotoxin (0.1 mg/kg) to control values at 4 h. N = 3 to 4, *$P < .0006$ vs control AFC; †$P < .0001$ vs LPS (0.1 mg/kg) AFC by ANOVA (Bonferroni). (*B*) Beneficial effect of the CM of human MSC on alveolar fluid clearance was mediated in part by KGF. Administration of the CM of MSC grown for 24 h pretreated with the KGF siRNA (Ambion) into the *E coli* endotoxin-injured lung lobe after 1 h prevented the restoration of AFC with the CM alone. The addition of recombinant KGF (100 ng) to the CM pretreated with KGF siRNA restored the decrease in AFC to control values. Data are expressed as mean AFC ± SD, N = 4 to 5 lungs, *$P < .0012$ vs control lobe by ANOVA (Bonferroni). AFC = alveolar fluid clearance. See Figures 1-3 legends for expansion of other abbreviations. (Reprinted from Lee et al.[17])

complications.[31] In patients with nonseptic ALI, mortality in one recent trial was 13%.[32]

For the initial evaluation of cell-based therapy for ALI, we believe that patients with a high risk of death should be enrolled. Higher mortality provides more opportunity for benefit and a more favorable risk-to-benefit profile should unanticipated toxicities appear. Although MSC administration has been safe in a number of human studies, we do not yet know the safety profile of administering MSC to patients with ALI, so there must be appropriate monitoring regarding both acute and longer-term safety issues. Therefore, it seems reasonable to test MSC therapy in patients in whom the risk of death is substantially higher than in current NHLBI ARDS Network trials.

How should patients be identified with a higher predicted mortality, using practical criteria that can be assessed efficiently and accurately? One approach would be to select a cutoff for a severity of illness score, similar to the Food and Drug Administration suggested use of Acute Physiology and Chronic Health Evaluation II values > 25 for patient selection for activated protein C treatment of severe sepsis. However, the use of a cutoff with a severity of illness score has major limitations.[33] Another approach would be to enroll patients with a more severe oxygenation defect. In one large population-based cohort, lower PaO_2/FIO_2 ratios were independently associated with mortality with a marked increase in the adjusted odds ratio for death when the PaO_2/FIO_2 fell to < 100 (odds ratio, 3.04; 95% CI, 1.74-5.31).[34] In 2,314 patients enrolled into prior ARDS network trials, mortality in the tertiles of baseline PaO_2/FIO_2 was 23% (>170), 28% (110-170), and 37% (<110, lowest tertile; P = .0001).[35] Because oxygenation can be substantially influenced by the level of positive end-expiratory pressure, it might be reasonable to require that the PaO_2/FIO_2 cut-off that requires at least 8 cm H_2O positive end-expiratory pressure. A third approach would be to enroll patients with multiorgan failure in whom systemic MSC therapy might benefit several different end-organs. For example, based on preclinical evidence, MSC may favorably affect both lung and kidney injury, and patients with ALI and acute kidney injury have an increased risk of death.[36] Therefore, especially if the systemic value of MSC is to be tested by IV administration of MSC, we would not exclude patients with ALI with renal dysfunction unless they already required dialysis. Another approach would be to include patients with ALI with shock as defined by the need for vasopressor therapy. In the ARDS network FACTT trial, one-third of the 1,000 patients had shock at baseline and a mortality of 35% to 41%.[37] These options are summarized in Table 1.

Table 1—Potential Inclusion Criteria for the Initial Trial of Allogeneic Human Mesenchymal Stem Cells for Acute Lung Injury/ARDS

Bilateral infiltrates in the chest radiographs and

1. PaO_2/FIO_2 < 200 on at least 8 cm water positive end-expiratory pressure or[a]
2. PaO_2/FIO_2 < 300 plus the need for vasopressor support (excludes dopamine < 6 mg/kg/min)[a]

[a]Patients with elevated serum creatinine would not be excluded unless they already required dialysis.

Which risk factors for ALI should be required for patient selection? Because attributable mortality from trauma-related ALI has decreased in recent years to 12% to 13%, we would exclude trauma-related ALI.[38] Based on preclinical studies, infection-related ALI would be the most important risk factor, including both pulmonary and nonpulmonary infections. Aspiration of gastric contents could be included as well, especially because it is likely that part of the mechanism of ALI in gastric aspiration can be explained by the presence of aerobic and anaerobic organisms in the aspirated contents.

It would also be important that the eligible patients do not have comorbidities or demographic factors that would limit the enthusiasm of physicians and the family for cell-based therapy. In a clinical trial of intensive insulin therapy for sepsis, the loss of patients from the trial because of withdrawal of life support within 72 h of enrollment appeared to confound the potential benefit of the intensive insulin therapy.[39] Enrollment should be accomplished within 48 to 72 h after the onset of severe ALI to maximize the potential for MSC to modulate pathologic inflammation and limit tissue injury.

Route of Delivery

The optimal route of delivery for the MSC is unknown. Would IV delivery of MSC result in the same benefit as direct intrapulmonary intraalveolar delivery? MSC do home to injured organs, whether the injury occurs in the liver, kidney, or the lung.[1] The efficacy of the IV vs the intraalveolar route is being tested in our ex vivo perfused human lung preparation and in small animal models of ALI. Preliminary evidence indicates that the IV route is as effective as the intraalveolar route.[40] Given the higher complexity of intraalveolar administration, the patchy nature of ALI, and because MSC have been administered IV with a good safety record, parenteral dosing via the IV route might be the preferred approach. MSC would reach the pulmonary microcirculation first, although some MSC would still circulate to other organs that are often injured in patients with ALI. For example, mortality increases from 24% to 58% in

patients with ALI who develop acute kidney injury after they have developed ALI.[36] Thus, IV dosing might provide better therapy to systemic organs as well as the lung.

Dose and Timing of Treatment

What would be the ideal timing for administration of MSC in severe ALI? Currently, we would favor treatment on three consecutive days. This choice would provide treatment during the acute phase of severe ALI. Dr. Duncan Stewart has used a 3-day time period for administering autologous endothelial progenitor cells in patients with end-stage pulmonary hypertension.[41] In an initial clinical trial, it would be reasonable to test two different doses of MSC. The final dose selection should be guided by our preclinical work and by MSC trials that have been carried out for other conditions. For instance, in two phase III trials using Prochymal (adult allogeneic mesenchymal stem cells) in severe refractory graft-vs-host disease, Osiris Therapeutics used MSC dosage of 2 million cells/kg twice a week for 4 weeks. The trial was inspired by positive phase II results, which showed an overall response rate of 94% and complete remission rate of 77% among 32 patients who received MSC for acute graft-vs-host disease (NCT00366145, NCT00562497).

How To Assess Safety in a Clinical Trial

One important purpose of an initial trial would be to assess safety of MSC therapy in patients with severe ALI. Because all patients will be intubated and mechanically ventilated in an ICU, continuous monitoring of the heart rate, rhythm, and systemic BP, and frequent recording of ventilator parameters and organ function will be possible and part of their ongoing clinical care. Continuous monitoring of vital signs, vasopressor doses, and oximetry, as well as periodic recording of airway pressures and dead space fraction, could be done during and for 2 h after IV administration to be sure that there was no evidence of acute hemodynamic instability from sequestration of MSC in the pulmonary microcirculation. Close monitoring for acute cardiovascular or hypersensitivity reactions would continue for the first 2 weeks. In keeping with recent recommendations for clinical trials of immune-modulating therapy in the wake of the anti-CD28 monoclonal antibody experience (http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_063117), patients would be carefully monitored.[42] In the anti-CD28 monoclonal trial, six normal volunteers were given a humanized monoclonal antibody and all six individuals developed multisystem organ dysfunction with shock, respiratory failure, and renal failure, all requiring prolonged treatment in the ICU. The report is a sobering reminder to all investigators that the use of new therapies in patients must proceed with caution, close monitoring, and careful selection of the doses of the therapy with an appropriate interval of time to assess safety. Although MSC therapy has been well tolerated in patients to date, cell-based therapy has not been administered to patients with severe ALI before. Thus, it is critical to monitor safety, which can be better accomplished with a control group.

Additional monitoring and rigorous testing for longer-term safety would need to be carried out, including assessment of potential untoward effects related to suppression of the host immune response, such as bacterial, viral, or fungal infection, and other unanticipated responses or outcomes, including neoplastic transformation. Based on the some of the experimental data published so far, MSC do not appear to impair host defense against bacteria. In fact, the opposite seems to be the case: in preliminary studies from our laboratory, MSC therapy was associated with a reduction in the number of organisms in a mouse model of live *E coli* pneumonia. The mechanisms by which MSC provide an antibacterial effect will be important to identify as well.

How To Assess Efficacy in an Initial Clinical Trial?

What are the best approaches to assess efficacy clinically and biologically? An initial clinical trial cannot provide comprehensive data regarding efficacy. However, we believe that the initial clinical trial can be designed to study appropriate biologic and clinical end points, providing guidance for future clinical trials of MSC in severe ALI. We also favor a randomized control group for the initial trial to make the biologic and clinical data more understandable.

We propose to separate biomarkers into two groups. Group 1 would be markers of MSC activity in vivo (eg, KGF, angiopoietin-1, PGE-2, IL-1ra) that would be measured in the plasma and in the BAL fluid. Group 2 would reflect lung and systemic injury, to be measured also in plasma and in BAL. Thus, group 1 biomarkers would provide evidence for biologic activity of the MSC and the host interaction and group 2 biomarkers would function as end points for the degree of modulation of lung and systemic injury by MSC. The proposed group 2 biologic end points to assess the response to MSC therapy are excellent biologic markers of endothelial injury (Von Willebrand factor antigen, angiopoietin-2), alveolar epithelial injury (receptor for advanced end glycation products, surfactant protein D), acute inflammation (IL-6, IL-8), and indices of procoagulant and antifibrinolytic activity (protein C, thrombomodulin, tissue factor, plasminogen activator

inhibitor-1) that are elevated in ALI. Other biologic markers may be useful as well (Table 2).

The clinical end points would be focused on physiologic end points in an initial clinical trial. Clinical end points should include the oxygenation index because it incorporates two important pulmonary physiologic end points, oxygenation and mean airway pressure. Recent studies from both our group and the NHLBI ARDS Network have indicated that the oxygenation index has prognostic value for mortality[43] and also tracked well with the beneficial effects of a fluid-conservative therapy in ALI,[29] which resulted in more ventilator-free days. Additional clinical end points could include the 4-point ALI score as well as the pulmonary dead space fraction, another end point that has prognostic value in patients with ALI.[44,45] In addition to pulmonary physiologic end points, it will be important to measure nonpulmonary organ function in ARDS network trials. Table 3 summaries the potential clinical end points.

Conclusions

Preclinical studies suggest that MSC may have value for the treatment of ALI. Because overall mortality has declined in ALI with improved supportive care, we favor testing cell-based therapy in severely ill patients with ALI with a predicted mortality > 30%. For an initial clinical trial, the emphasis needs to

Table 3—Clinical End Points for an Early Clinical Trial of Allogeneic Human Mesenchymal Stem Cells for ARDS

Oxygenation index
Acute lung injury score
Ventilator-free days
Duration of shock
Need for dialysis/nonpulmonary organ failures
Mortality

be on well-defined safety and clinical end points, including biologic and clinical criteria.

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