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Mesenchymal Stem Cells and Acute Lung Injury

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Acute respiratory distress syndrome (ARDS) was first recognized in the 1960s¹ as a clinical syndrome of severe acute respiratory failure presenting with hypoxemia and bilateral pulmonary infiltrates, most often in the setting of pneumonia, sepsis, or major trauma. The distinction between acute lung injury (ALI) and ARDS relates to the severity of hypoxemia, with the former having a $\text{PaO}_2/\text{FiO}_2 < 300$, and the latter with a $\text{PaO}_2/\text{FiO}_2 < 200$. The pathogenesis of ALI/ARDS involves lung endothelial injury, alveolar epithelial injury, and the accumulation of protein-rich fluid and cellular debris in the alveolar space (for recent review see Matthay and Zemans²). In 2005, approximately 200,000 patients in the United States developed ALI/ARDS, with an estimated mortality of 40%³. In the era of lung protective ventilation, mortality has declined to approximately 25%⁴. However, of the broad array of pharmacologic therapies evaluated in clinical trials to date, including inhaled surfactant, nitric oxide, prostacyclins, glucocorticoids, ketoconazole, antioxidants, β -agonists, and pentoxifylline, none has proven effective, and none can be currently recommended as standard therapy for ALI.⁵ Some may have value as rescue therapies⁶. It is possible that the lack of efficacy with pharmacologic therapies is in part due to the relatively late stage at which therapy is initiated as well as the heterogeneity of ALI, with somewhat different pathophysiologic cascades predominating in ALI due to trauma versus infectious causes of ALI⁷. Given these challenges, and with severe pathologic changes at the level of the alveolus in ALI, it seems increasingly unlikely that any single molecule will prove sufficient to reverse the course of this syndrome rapidly enough to provide substantial clinical benefit.

A potential strategy to circumvent these difficulties involves cell-based therapies. Given the ability of some cells to produce dozens of active molecules that can potentially modulate inflammatory cascades at multiple levels as well as enhance repair, it is conceivable that such therapies would prove more successful than single molecules. A steadily enlarging body of evidence from preclinical studies of lung injury, sepsis, and other disease states indicates that one ideal candidate may be the mesenchymal stem cell.

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Mesenchymal Stem Cells--General Properties

Mesenchymal stem cells (MSCs) are multipotent, self-renewing cells initially isolated from bone marrow that can differentiate into muscle, bone, fat, fibroblasts, and cartilage. They were first described in the late 1960s⁸ when it was discovered that a fraction of cells from whole bone marrow adhered to plastic. These cells were spindle-like, formed colonies, could reconstitute a hematopoietic environment, and could regenerate bone tissue in serial transplants, demonstrating their ability to self-renew. Though initially termed Colony-Forming Unit-Fibroblastic (CFU-F), these cells were later referred to as marrow stromal cells, and ultimately were labeled “mesenchymal stem cells” by Caplan and colleagues.⁹ The lack of specific cell markers has slowed their *in vivo* characterization. It is still not known, for example, whether MSCs originate from the mesoderm, from the neuroepithelium, or from different sources at progressive developmental stages as a recent study has suggested.¹⁰ It is now generally accepted that MSCs or MSC-like cells can be isolated not just from bone marrow, but also from fat, umbilical cord blood, placental tissue, tendons, and skeletal muscle.¹¹¹²¹³¹⁴¹⁵ In the absence of cell-specific markers, the following criteria have been put forth by the International Society of Cellular Therapy:¹⁶ (a) adherence to plastic, (b) expression of CD105, CD73, and CD90; lack of expression of CD45, CD34, CD14, CD11b, CD79a, CD19, HLA II, and (c) ability to differentiate into osteoblasts, adipocytes, and chondroblasts *in vitro*.

Based on their differentiation capacity, researchers have studied these cells for their potential to repair damaged musculoskeletal tissues. However, MSCs are now known to possess multiple other properties that have galvanized the scientific community. MSCs expanded *in vitro* home to sites of tissue damage.¹⁷¹⁸ Though a full review of their properties is beyond the scope of this article, some will be considered briefly here. MSCs produce a wide variety of molecules, including hematopoietic factors, chemokines, and angiogenic factors.¹⁹ Given their presence in the bone marrow, it is not surprising to learn that they have potent immunomodulatory effects. Generally speaking, they shift the immune response toward tolerant, anti-inflammatory phenotypes.²⁰ For the most part they lack MHC Class II antigens, and consequently can evade immune destruction following allogeneic or even xenogeneic transplant. For this reason, MSCs have been developed as vectors for gene therapy, and to help induce tolerance in allogeneic bone marrow transplantation.²¹ MSCs have now been found to have beneficial effects in preclinical models of diseases, ranging from Crohn's disease²² to traumatic brain injury.²³ Over the last decade, several investigators have reported that exogenously administered MSCs can mitigate several types of lung disease in a variety of animal models.

MSCs in Lung Disease

Bleomycin exposure has previously been developed as a model of fibrotic lung disease in mice.²⁴²⁵ Ortiz and colleagues²⁶ isolated murine MSCs and administered them intravenously immediately or seven days following bleomycin injury. They found that exogenous MSCs could be found in areas of bleomycin-induced lung injury, and that these cells appeared to acquire characteristics of epithelial cells. Mice treated with MSCs immediately following bleomycin exposure also had significantly reduced collagen deposition, and reduced expression of matrix metalloproteinases 2 and 9. The degree of the anti-inflammatory effects were striking in comparison to the relatively low levels of lung engraftment. In subsequent work²⁷, this group reported that MSC *in vivo* administration blocks the bleomycin-induced increase in IL-1 α . Rojas et al.²⁸ administered bleomycin to mice with or without preceding busulfan-induced myelosuppression. Myelosuppression enhanced the initial injury and was associated with reduced survival. However, myelosuppressed mice that received GFP-tagged MSCs six hours after bleomycin injury had improved survival, an effect that was

associated with engraftment of GFP-positive cells that expressed markers of fibroblasts, myofibroblasts, as well as type I and type II alveolar epithelial cells. Similarly, Moodley and colleagues²⁹ derived MSCs from umbilical cord tissue and infused them intravenously following nasal bleomycin in mice. MSCs were identified two weeks later in inflamed portions of the lung. MSCs reduced collagen concentration as well as Smad2 phosphorylation, suggesting that these cells had antifibrotic properties.

In a model of bronchopulmonary dysplasia, Chang et al.³⁰ delivered MSCs intratracheally in postnatal rats exposed to prolonged hyperoxia. The MSCs significantly reduced apoptosis, myeloperoxidase activity, and collagen deposition, as well as the inflammatory molecules IL-6, TNF- α , and TGF- β . Furthermore, a few of the MSCs differentiated into type II alveolar epithelial cells, though as in many such studies, at a low level. In similar studies with postnatal rats exposed to prolonged hyperoxia, van Haaften et al.³¹ reported that intratracheal MSCs improved survival and exercise tolerance, and decreased alveolar and vascular lung injury, as well as pulmonary hypertension in a neonatal model of lung injury.

MSCs, delivered intratracheally, reduced pulmonary hypertension induced by monocrotaline in rats, and improved measures of vascular endothelial function.³² In a rat model of emphysema induced by irradiation and papain, Zhen and colleagues³³ reported that MSC administration reduced emphysematous changes. Additionally, MSCs differentiated into type II alveolar epithelial cells. Thus, MSCs have therapeutic effects in several models of lung disease. Because of their anti-inflammatory properties, they would be especially well-suited to mitigate the lung damage in ALI.

MSCs in animal models of ALI and sepsis—Evidence of Beneficial Effects

Lipopolysaccharide (LPS) has been widely used to produce acute lung injury in animal models. When given by the airway, LPS triggers a large influx of PMNs into the airspaces that peaks around 48 hours and is associated with increased microvascular permeability.³⁴ A number of investigators have studied the properties of MSCs in LPS-induced models of acute lung injury. Yamada and colleagues³⁵ delivered LPS intranasally and showed that MSCs were increased in peripheral blood four hours later. They next subjected irradiated mice with bone marrow reconstituted from GFP transgenic donors to intranasal LPS, and demonstrated abundant GFP-positive cells in the lungs three weeks later. Some of these cells expressed cytokeratin, a marker of epithelial cells, while others expressed CD34, a marker of endothelial cells. These results suggested that endogenous MSCs might play an important role in repairing inflammatory damage following LPS. Interestingly, following sublethal irradiation to induce bone marrow suppression, intranasal LPS produced a similar pattern of histological damage and BAL neutrophilia, but at one week, large emphysematous-like lesions were noted. These lesions could be prevented by marrow reconstitution, suggesting the absence of endogenous MSCs may have compromised normal repair processes.

Mei et al.³⁶ infused MSCs intravenously 30 minutes after intratracheal LPS, showing a significant reduction in BAL total cell and neutrophil counts three days later. Histological analysis confirmed a marked decrease in inflammatory infiltrates, interalveolar septal thickening, and interstitial edema. Using fluorescent tags for MSCs, these researchers demonstrated many labeled cells in the lungs of both LPS-injured and control animals 15 minutes after cell infusion, with a few labeled cells persisting at 3 days.

Xu et al.³⁷ used intraperitoneal LPS at 1 mg/kg (a dose which causes minimal mortality), and one hour later infused MSCs or fibroblasts intravenously. Histological analysis revealed that MSCs, but not fibroblasts, significantly reduced lung neutrophils at 6, 24, and 48 hours. Seeking to model a more realistic clinical time course and a more critical illness, Gupta and colleagues³⁸ administered MSCs intratracheally to mice 4 hours following intratracheal

administration of 5 mg/kg LPS, a dose which produces significant mortality. MSC-treated mice had improved survival relative to PBS-treated mice: 80% vs. 42% at 48 hours, and 64% vs. 18% at 72 hours. MSC reduced the severity of lung injury as measured by excess lung water, wet-to-dry ratio, and BAL protein concentration. Histological analysis at 48 hours revealed less hemorrhage and edema. Nonviable MSCs and fibroblasts did not replicate this effect, suggesting undifferentiated, viable MSCs were required to ameliorate LPS-induced ALI. Recent work from Krasnodembskaya and colleagues³⁹ extended this work in a model of *E-coli* pneumonia. *E-coli* was administered to mice intratracheally. Four hours later these mice were treated with intratracheal MSCs, PBS, or fibroblasts. MSCs substantially reduced lung inflammation, as measured by BAL neutrophil count.

Mei et al.⁴⁰ studied a cecal ligation and puncture (CLP) model of sepsis in mice. Six hours following CLP, MSCs or saline was infused intravenously. All mice received daily broad-spectrum antibiotics. The authors found that MSC-treated mice had decreased BAL cell counts and albumin. Histology confirmed reduced inflammatory lung infiltrates and interstitial edema at 28 hours. Interestingly, the therapeutic benefit of MSCs was not confined to the lungs, as MSC-treated mice had reduced apoptotic kidney cells and improved serum creatinine. CLP results in severe systemic injury, as evidenced by 45% mortality at 28 hours. Remarkably, MSC-treatment improved mortality by 50% at this time point.

Finally, Nemeth and colleagues⁴¹ administered IV MSCs or control cells (heat-killed MSCs or skin fibroblasts) 24 hours before or one hour after CLP in mice. All mice received subcutaneous isotonic fluid and broad-spectrum antibiotics. They demonstrated a substantial survival benefit for MSCs (with both administration times) at 4 days. MSCs from multiple different strains of mice provided an equivalent survival benefit for the C57BL/6 recipients. MSC-treated mice also had lower serum creatinine and kidney tubular injury scores, improved hepatic glycogen storage and reduced transaminases, amylase, and splenic apoptosis, suggesting multiple beneficial systemic effects. Most of these effects were observed in mice treated with MSCs 24 hours before CLP.

MSCs in animal models of ALI and sepsis—Potential Mechanisms

Over the last several years, investigators have examined how MSCs may exert their therapeutic effects in models of ALI and sepsis, and have discovered an increasing number of potential mechanisms. It is helpful first to consider the processes governing the production and removal of alveolar edema fluid.

Endothelial Permeability, Epithelial Permeability, and Alveolar Fluid Clearance

Edema accumulates in the alveoli through some combination of increased permeability to protein of the endothelial and epithelial barriers, and reduced (or insufficient) alveolar fluid clearance (AFC). A number of groups have reported that MSCs reduce the increase in endothelial permeability associated with ALI. BAL albumin and protein are commonly used as markers of lung endothelial permeability, though in reality reflect a combination of endothelial and epithelial permeability. Mei and colleagues³⁶ found that BAL albumin, total protein, and IgM were increased 3 days following intratracheal LPS. This increase was attenuated by MSCs given intravenously 30 minutes after the injury. Angiopoietin-1 (Ang-1) may help maintain adult vascular endothelial cells in a quiescent state, and Ang-1 has been shown to reduce permeability and promote endothelial cell survival.⁴²⁴³⁴⁴ Mei and colleagues reported that MSCs engineered to produce angiopoietin-1 (Ang-1) further reduced BAL protein, albumin, and IgM to levels in uninjured mice. They reasoned that Ang-1 acted on the vascular endothelium following delivery of MSCs into the pulmonary circulation, diminishing inflammatory cell influx and reducing plasma protein leakage into

the alveolar space. In a similar set of experiments, Xu et al.⁴⁵ showed that MSCs overexpressing Ang-1 delivered intravenously on the 1st day of 7 days of nebulized LPS reduced BAL protein 7 and 14 later. More recently, Mei et al.⁴⁰ reported that IV infusion of MSCs 6 hours following cecal ligation and puncture (CLP) reduced BAL protein and albumin 28 hours post-injury, suggesting MSCs reduce the endothelial permeability associated with sepsis as well. This was further supported by the work from Nemeth et al.⁴¹ These researchers showed that 24 hours following CLP in mice, Evans blue dye leakage was reduced by IV MSC administration in multiple organs, including the lung, liver, and kidney. Krasnodembskaya et al.³⁹ tested the effects of MSC administration in a more biologically relevant model of pneumonia. They reported that mice given live *E-coli* intratracheally had elevated BAL protein 18 hours later, and that BAL protein was significantly decreased by intratracheal delivery of MSCs (but not fibroblasts) four hours after the injury.

As indicated above, measurements of BAL protein provide an estimate of the combined changes in lung endothelial permeability, epithelial permeability, and AFC. The alveolar epithelium normally forms a tighter barrier than the endothelium, and its loss of integrity in ALI is of great significance. It had previously been shown that pulmonary edema fluid from patients with ALI increased epithelial protein permeability in primary cultures of human alveolar type II (ATII) cells grown on a semi-permeable membrane with an air-liquid interface.⁴⁶ Fang et al.⁴⁷ sought to study the effect of MSCs on the alveolar epithelium using this model. When exposed to a potent mixture of inflammatory cytokines (cytomix, composed of IL-1 β , TNF- α , and IFN- γ), protein permeability across the epithelial layer increased by approximately 500%. However, when co-cultured with allogeneic MSCs (in the lower compartment of a Transwell[®] system, precluding cell contact), protein permeability was reduced to control levels. Angiopoietin-1, shown in several in vivo studies to augment the beneficial effect of MSCs (see above), was not produced by ATII cells at baseline or in response to cytomix. However, MSCs secreted Ang-1, and appeared to augment its production in response to cytomix. When Ang-1 production was blocked by siRNA, MSCs no longer prevented cytomix-induced increased epithelial permeability. However, the effect was restored by the addition of recombinant Ang-1. In an additional series of experiments, Ang-1 acted on epithelial cells through a Tie-2 receptor mechanism involving NF- κ B to prevent the formation of actin stress fibers and maintain the localization of claudin-18, a key tight junction protein, at the cell periphery. These findings suggest that MSCs, acting in part through angiopoietin, improve the critical barrier function of the alveolar epithelium in the inflammatory milieu of ALI.

Alveolar fluid clearance (AFC) is the capacity of the epithelium of the lung to remove alveolar fluid in pulmonary edema of any etiology, and impaired AFC is associated with worsened outcomes in ALI/ARDS.^{48,49} Many conditions can reduce AFC, including high tidal volume ventilation, live bacteria, acid instillation, and pro-inflammatory cytokines.^{50,51,52,53,54} Gupta et al.³⁸ found that intratracheal MSCs 4 hours after LPS reduced excess lung water, though did not distinguish between changes in formation of fluid (via increased endothelial and epithelial permeability) and changes in AFC. Lee et al.⁵⁵ developed an ex vivo perfused human lung preparation, and tested the effects of allogeneic MSCs following intra-bronchial administration of LPS. In this model, LPS resulted in a marked decrease in AFC, from approximately 20% per hour to near 0% per hour, an effect that required the presence of blood in the perfusate, suggesting that blood elements such as PMNs, monocytes, and platelets were required for the injurious effect of LPS. Instillation of fibroblasts into the injured lung one hour after LPS had no effect on AFC. However, instillation of human MSCs or their conditioned medium normalized AFC to baseline levels. This effect appeared to be modulated in part by keratinocyte growth factor (KGF), as siRNA for KGF reduced the therapeutic effect of MSCs on AFC by 80%. Thus, MSCs appear to improve both endothelial/epithelial permeability and AFC in multiple models of ALI.

Engraftment—Though MSCs were initially noted to differentiate into bone, fat, muscle, and cartilage,⁵⁶ researchers discovered in the late 1990s that under certain conditions, these cells could develop a non-mesodermal phenotype.^{57,58} Kotton et al.⁵⁹ showed that labeled MSCs incorporated into lung tissue following bleomycin injury and developed morphological and molecular characteristics of type I pneumocytes. Xu and colleagues³⁷ noted large numbers of labeled MSCs in the lung parenchyma 24 hours following IP LPS and IV MSC infusion.

However, 2 weeks later very few labeled cells remained, suggesting the substantial presence of MSCs in the lung is a transient phenomenon following ALI. Gupta et al.³⁸ found scattered labeled MSCs at 24 and 48 hours following IT LPS and MSC delivery, with less than 5% engraftment. Xu and colleagues⁴⁵ treated mice daily for seven days with nebulized LPS, infusing GFP-labeled MSCs on the first day. In some animals, MSCs were also engineered to express angiopoietin-1, the ligand for Tie2 known to decrease endothelial permeability. Fourteen days after infusion, labeled cells were seen in the lung (reported 9% engraftment with MSCs and 16% with MSC-Ang1), though the engrafted cells were not further characterized. Taken together, the data suggest that MSC differentiation into mature lung cell types following ALI may occur at low levels but is unlikely to produce much of their beneficial effect.

Immunomodulation—MSCs were first recognized to have potent immunomodulatory effects based on their ability to engraft and differentiate following xenogeneic transplantation.⁶⁰ MSCs have since been shown to suppress many functions of naïve and memory T cells, B cells, NK cells, and the differentiation and function of monocytes.^{61,62,63} In many models of ALI, MSC administration results in altered signaling related to immune cell activation and recruitment. In their intratracheal LPS model, Gupta et al.³⁸ reported that intratracheal MSCs reduced BAL levels of the proinflammatory cytokines TNF- α and MIP-2 as well as plasma levels of MIP-2. There was a corresponding increase in the anti-inflammatory cytokines IL-10, IL-1 α , and IL-13. Furthermore, the addition of MSCs to LPS-stimulated macrophages in vitro reduced the level of TNF- α , an effect that persisted in the presence of a Transwell[®] that prevented contact between the cell types. These findings suggest that the administration of MSCs in a model of ALI shifts the injured lung milieu from proinflammatory to anti-inflammatory, in part through effects of soluble mediators on macrophages.

Xu et al.³⁷ showed that intravenous MSCs moderated the LPS-induced increase in serum proinflammatory IFN- γ , IL-1 β , macrophage inflammatory protein 1- α , and KC (murine homolog of IL-8). In their cecal ligation and puncture (CLP) model, Nemeth and colleagues⁴¹ reported that intravenous MSCs reduced serum TNF- α and IL-6 but increased IL-10 24 hours post-injury. They also found that MSCs reduced myeloperoxidase in the liver and kidneys of LPS-treated mice, suggesting less neutrophil invasion and associated organ damage. They then performed a series of experiments to help elucidate the molecular signaling driving these effects. MSCs improved survival in mice genetically lacking mature T and B cells, and in mice depleted of NK cells. Next they depleted mice of monocytes and macrophages and found that the MSCs were no longer effective, suggesting that these were the cells through which MSCs exert their beneficial effects. In vitro, MSCs were shown to increase COX2 expression and activity within 5 hours of LPS stimulation, followed by elevated levels of prostaglandin E2 (PGE2), effects that were abolished in MSCs taken from TLR4 knockout mice or in the presence of antibodies to TNF- α . MSCs were then cultured with macrophages, and the addition of LPS was found to increase IL-10. In a series of experiments, this effect was shown to depend on TLR4, MyD88 (required for NF- κ B activation by TLR4), TNF- α and TNF receptor-1. In additional experiments, they demonstrated that the macrophages responded to PGE2 via EP2 and EP4 receptors. Thus

they concluded that MSCs activated by LPS reprogram macrophages to secrete IL-10, and that this reduces neutrophil migration into tissues and helps mitigate tissue damage.

Mei et al.⁴⁰ reported that 28 hours following CLP, mice treated with MSCs (given IV 6 hours after injury) had diminished serum levels of the proinflammatory cytokines, IL-6, IL-1 β , KC (murine homolog of IL-8), JE (murine homolog of MCP-1), and CCL5 (murine homolog of RANTES). Serum IL-10 was also decreased by MSCs in this model, in contrast to the previous study by Nemeth et al.⁴¹ It is unclear why IL-10 was increased in one model of CLP and decreased in another, but this may relate to technical differences such as the timing of MSC administration relative to injury or the number of cells infused. In any case, as became more clear following a gene expression analysis in this model (see below), it appears likely that MSC administration simultaneously modulates several inflammatory pathways rather than acting through any single mediator.

Antibacterial effects—It had been somewhat puzzling that MSCs could have such potent anti-inflammatory effects and yet lead to robust improvements in survival following sepsis induced by live bacteria. Some exciting recent experiments suggest that this appears to be due in part to MSCs ability to reduce the bacterial burden. As described above, Mei et al.⁴⁰ gave intravenous MSCs 6 hours following cecal ligation and puncture and found a significant improvement in survival. They compared bacterial CFU from the spleen 28 hours after injury, and found that MSC treatment reduced mean CFU by an order of magnitude. Next they isolated total cells or the CD11b+ fraction (monocytes/macrophages and neutrophils) of cells from the peritoneal space and spleens of mice 24 hours after CLP. They showed that both the total cell population and the CD11b+ fraction had increased phagocytic capacity for gram-negative and gram-positive aerobic bacteria following MSC treatment. Additional experiments suggested that MSCs themselves infrequently engaged in phagocytosis. This suggested that they must indirectly modulate the host's phagocytes. Interestingly, in a gene expression analysis of splenic tissue 28 hours after CLP, MSC treatment down-regulated genes involved in inflammatory pathways such as IL-6, IL-1, the IL-1 receptor, and IL-10. Conversely, MSCs tended to up-regulate genes involved in phagocytosis.

Krasnodembskaya et al.³⁹ recently reported that MSCs possess additional antimicrobial properties. These researchers first showed that MSCs (compared to fibroblasts) reduced the growth of *E.coli* in vitro. MSC-conditioned medium had no effect on gram-negative bacterial growth unless the cells had previously been stimulated with *E.coli*, suggesting that this induced them to produce an antibacterial substance. They next screened the media of bacteria-stimulated MSCs for known antimicrobial peptides and proteins, and found significant quantities of human cathelicidin (LL-37). Synthetic LL-37 reduced the growth of *E.coli* and *P.aeruginosa*, and when incubated with a blocking antibody for LL-37, the conditioned medium of bacterial-stimulated MSCs lost its antibacterial effect. When mice were given *E.coli* intratracheally and then treated with MSCs four hours later, both lung homogenates and BAL showed over an order of magnitude reduction in bacterial counts. This reduction was largely prevented by coadministration a neutralizing antibody to LL-37. That BAL neutrophil counts were similarly reduced by MSC treatments suggested that improved bacterial clearance was not dependent on this cell type. Finally, the BAL from MSC treated mice itself inhibited bacterial growth. Taken together, the results suggest that MSCs exert both direct effects on bacteria and positively modulate the host's phagocytic capacity. Future experiments should help clarify the precise cellular and molecular pathways of phagocytic augmentation, and may identify additional direct antibacterial effects.

Other possible mechanisms—A number of recent studies have also revealed entirely new mechanisms of interaction between MSCs and tissue cells. Spees et al.⁶⁴ depleted

functional mitochondria in A549 cells using ethidium bromide to mutate mitochondrial DNA. Interestingly, when subsequently cultured with MSCs, the A549 cells acquired functional mitochondria whose DNA matched that of the MSCs. Time-lapse microscopy showed MSCs develop extensions of their cytoplasm toward the A549 cells through which mitochondria subsequently streamed. This report did not establish the ultrastructural mechanism of mitochondrial transfer. More recently, Plotnikov and colleagues⁶⁵ demonstrated *in vitro* mitochondrial transfer from MSCs to cardiac myocytes. Electron microscopy revealed extremely thin structures termed nanotubes, through which the mitochondria appeared to travel. Whether the mitochondria travel by vesicles, nanotubes, or some other mechanism, the observation that MSCs can rescue energetically compromised cells by directly transferring their mitochondria suggests another potential beneficial role *in vivo*, as mitochondrial compromise is a common feature of many models of organ injury.

Microvesicles (MV) are vesicles/exosomes released by multiple types of cells, including stem cells, and have recently been recognized to be an important mechanism of communication.^{66,67} Embryonic stem cells have been shown to reprogram hematopoietic progenitors by mRNAs carried in microvesicles.⁶⁸ MSCs appear to release microvesicles as well. Bruno and colleagues⁶⁹ showed that MSC-derived microvesicles increased proliferation of kidney tubular epithelial cells *in vitro*, and made them resistant to apoptosis in response to serum deprivation or administration of vincristine and *cis*-platinum. Next they showed that MSCs or their microvesicles, when given to SCID mice, had similar protective effects against acute kidney injury caused by glycerol. Interestingly, RNase abolished the positive effect of microvesicles *in vitro* and *in vivo*, suggesting the effect was due to MSC-derived RNAs. Gene chip analysis of MSC microvesicles revealed mRNAs associated with transcription, proliferation, and immune cell regulation. More recent work from this same group has shown that MSC-derived microvesicles contain microRNAs as well.⁷⁰ What role microvesicles or mitochondrial transfer may play in the beneficial effects of MSCs in models of ALI remains to be determined.

Future studies

Clearly much about MSC biology remains to be discovered. Already these cells are known to exert a wide range of effects through an impressive and growing array of mechanisms. Future work should help clarify the relative contributions of engraftment, immunomodulation, antibacterial effects, mitochondrial transfer, and microvesicular transfer of genetic information. Some additional basic questions remain. The lack of reliable cell surface markers for these cells has significantly limited *in vivo* study. We have no knockout mouse for MSCs, no MSC specific promoters or immunohistochemical markers. This has left open the question of where MSCs can be found outside the bone marrow, with some groups suggesting that they may be equivalent to pericytes, which line most vascular elements in the body.^{71,72} And though the spectrum of beneficial effects from exogenous MSC administration is impressive, we have little sense of how native MSCs respond to injury, or whether there are ways to augment these responses, short of supplying them exogenously with potential risks to patients. We also need to know how much of their beneficial effects *in vivo* depend on paracrine factors and cell contact independent pathways versus cell contact-dependent mechanisms.

There has been enough promising preclinical data in a variety of disease states that human clinical trials have begun. MSCs are currently being studied in acute myocardial infarction, dilated cardiomyopathy, Crohn's Disease, chronic obstructive pulmonary disease, stroke, multiple sclerosis, acute graft versus host disease, type I diabetes mellitus, diabetic foot ulcer cirrhosis, and immune reconstitution syndrome in HIV (<http://clinicaltrials.gov>). The safety record for these cells has been reassuring to date.⁷³ However, there have not yet been

any clinical trials of MSCs in ALI or sepsis, despite the promising work outlined in this article. It is likely that this will change soon. The spectrum of possible MSC-based therapies for ALI includes both targeted intrapulmonary and intravenous administration, as well as any number of genetic modifications to these versatile cells. Further discussion on possible clinical trials can be found in a recent review.⁷⁴

References

1. Ashbaugh DG, Bigelow DB, Petty TL, Levine BE. Acute respiratory distress in adults. *Lancet*. 1967; 2(7511):319–323. [PubMed: 4143721]
2. Matthay, MA.; Zemans, RL. [Accessed November 3, 2010] The Acute Respiratory Distress Syndrome: Pathogenesis and Treatment. *Annu Rev Pathol*. 2010. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/20936936>
3. Rubenfeld GD, Caldwell E, Peabody E, et al. Incidence and outcomes of acute lung injury. *N Engl J Med*. 2005; 353(16):1685–1693. [PubMed: 16236739]
4. Wheeler AP, Bernard GR, Thompson BT, et al. Pulmonary-artery versus central venous catheter to guide treatment of acute lung injury. *N Engl J Med*. 2006; 354(21):2213–2224. [PubMed: 16714768]
5. Cepkova M, Matthay MA. Pharmacotherapy of acute lung injury and the acute respiratory distress syndrome. *J Intensive Care Med*. 2006; 21(3):119–143. [PubMed: 16672636]
6. Diaz JV, Brower R, Calfee CS, Matthay MA. Therapeutic strategies for severe acute lung injury. *Crit Care Med*. 2010; 38(8):1644–1650. [PubMed: 20562704]
7. Calfee CS, Eisner MD, Ware LB, et al. Trauma-associated lung injury differs clinically and biologically from acute lung injury due to other clinical disorders. *Crit Care Med*. 2007; 35(10):2243–2250. [PubMed: 17944012]
8. Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. *Transplantation*. 1968; 6(2):230–247. [PubMed: 5654088]
9. Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol*. 2007; 213(2):341–347. [PubMed: 17620285]
10. Takashima Y, Era T, Nakao K, et al. Neuroepithelial cells supply an initial transient wave of MSC differentiation. *Cell*. 2007; 129(7):1377–1388. [PubMed: 17604725]
11. Bi Y, Ehrichtou D, Kilts TM, et al. Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. *Nat Med*. 2007; 13(10):1219–1227. [PubMed: 17828274]
12. Bieback K, Klüter H. Mesenchymal stromal cells from umbilical cord blood. *Curr Stem Cell Res Ther*. 2007; 2(4):310–323. [PubMed: 18220915]
13. Crisan M, Yap S, Casteilla L, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell*. 2008; 3(3):301–313. [PubMed: 18786417]
14. Igura K, Zhang X, Takahashi K, et al. Isolation and characterization of mesenchymal progenitor cells from chorionic villi of human placenta. *Cytotherapy*. 2004; 6(6):543–553. [PubMed: 15770794]
15. Xu Y, Malladi P, Wagner DR, Longaker MT. Adipose-derived mesenchymal cells as a potential cell source for skeletal regeneration. *Curr Opin Mol Ther*. 2005; 7(4):300–305. [PubMed: 16121695]
16. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006; 8(4):315–317. [PubMed: 16923606]
17. Mansilla E, Marin GH, Sturla F, et al. Human mesenchymal stem cells are tolerized by mice and improve skin and spinal cord injuries. *Transplant Proc*. 2005; 37(1):292–294. [PubMed: 15808623]
18. Ries C, Egea V, Karow M, et al. MMP-2, MT1-MMP, and TIMP-2 are essential for the invasive capacity of human mesenchymal stem cells: differential regulation by inflammatory cytokines. *Blood*. 2007; 109(9):4055–4063. [PubMed: 17197427]

19. Kode JA, Mukherjee S, Joglekar MV, Hardikar AA. Mesenchymal stem cells: immunobiology and role in immunomodulation and tissue regeneration. *Cytotherapy*. 2009; 11(4):377–391. [PubMed: 19568970]
20. Ghannam S, Bouffi C, Djouad F, Jorgensen C, Noël D. Immunosuppression by mesenchymal stem cells: mechanisms and clinical applications. *Stem Cell Res Ther*. 2010; 1(1):2. [PubMed: 20504283]
21. Le Blanc K, Rasmusson I, Sundberg B, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet*. 2004; 363(9419):1439–1441. [PubMed: 15121408]
22. Valcz, G.; Krenács, T.; Sipos, F., et al. [Accessed October 30, 2010] The Role of the Bone Marrow Derived Mesenchymal Stem Cells in Colonic Epithelial Regeneration. *Pathol Oncol Res*. 2010. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/20405350>
23. Harting MT, Jimenez F, Xue H, et al. Intravenous mesenchymal stem cell therapy for traumatic brain injury. *J Neurosurg*. 2009; 110(6):1189–1197. [PubMed: 19301973]
24. Bowden DH. Unraveling pulmonary fibrosis: the bleomycin model. *Lab Invest*. 1984; 50(5):487–488. [PubMed: 6201673]
25. Harrison JH, Lazo JS. High dose continuous infusion of bleomycin in mice: a new model for drug-induced pulmonary fibrosis. *J Pharmacol Exp Ther*. 1987; 243(3):1185–1194. [PubMed: 2447265]
26. Ortiz LA, Gambelli F, McBride C, et al. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc Natl Acad Sci US A*. 2003; 100(14):8407–8411.
27. Ortiz LA, Dutreil M, Fattman C, et al. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. *Proc Natl Acad Sci US A*. 2007; 104(26):11002–11007.
28. Rojas M, Xu J, Woods CR, et al. Bone marrow-derived mesenchymal stem cells in repair of the injured lung. *Am J Respir Cell Mol Biol*. 2005; 33(2):145–152. [PubMed: 15891110]
29. Moodley Y, Atienza D, Manuelpillai U, et al. Human umbilical cord mesenchymal stem cells reduce fibrosis of bleomycin-induced lung injury. *Am J Pathol*. 2009; 175(1):303–313. [PubMed: 19497992]
30. Chang YS, Oh W, Choi SJ, et al. Human umbilical cord blood-derived mesenchymal stem cells attenuate hyperoxia-induced lung injury in neonatal rats. *Cell Transplant*. 2009; 18(8):869–886. [PubMed: 19500472]
31. van Haaften T, Byrne R, Bonnet S, et al. Airway delivery of mesenchymal stem cells prevents arrested alveolar growth in neonatal lung injury in rats. *Am J Respir Crit Care Med*. 2009; 180(11):1131–1142. [PubMed: 19713449]
32. Baber SR, Deng W, Master RG, et al. Intratracheal mesenchymal stem cell administration attenuates monocrotaline-induced pulmonary hypertension and endothelial dysfunction. *Am J Physiol Heart Circ Physiol*. 2007; 292(2):H1120–1128. [PubMed: 16980338]
33. Zhen G, Liu H, Gu N, et al. Mesenchymal stem cells transplantation protects against rat pulmonary emphysema. *Front Biosci*. 2008; 13:3415–3422. [PubMed: 18508443]
34. Chignard M, Balloy V. Neutrophil recruitment and increased permeability during acute lung injury induced by lipopolysaccharide. *Am J Physiol Lung Cell Mol Physiol*. 2000; 279(6):L1083–1090. [PubMed: 11076798]
35. Yamada M, Kubo H, Kobayashi S, et al. Bone marrow-derived progenitor cells are important for lung repair after lipopolysaccharide-induced lung injury. *J Immunol*. 2004; 172(2):1266–1272. [PubMed: 14707105]
36. Mei SHJ, McCarter SD, Deng Y, et al. Prevention of LPS-induced acute lung injury in mice by mesenchymal stem cells overexpressing angiopoietin 1. *PLoS Med*. 2007; 4(9):e269. [PubMed: 17803352]
37. Xu J, Woods CR, Mora AL, et al. Prevention of endotoxin-induced systemic response by bone marrow-derived mesenchymal stem cells in mice. *Am J Physiol Lung Cell Mol Physiol*. 2007; 293(1):L131–141. [PubMed: 17416739]

38. Gupta N, Su X, Popov B, et al. Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice. *J Immunol.* 2007; 179(3):1855–1863. [PubMed: 17641052]
39. Krasnodembskaya, A.; Song, Y.; Fang, X., et al. [[Accessed October 30, 2010]] Antibacterial Effect of Human Mesenchymal Stem Cells is Mediated in Part from Secretion of the Antimicrobial Peptide LL-37. *Stem Cells.* 2010. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/20945332>
40. Mei SHJ, Haitsma JJ, Dos Santos CC, et al. Mesenchymal stem cells reduce inflammation while enhancing bacterial clearance and improving survival in sepsis. *Am J Respir Crit Care Med.* 2010; 182(8):1047–1057. [PubMed: 20558630]
41. Németh K, Leelahavanichkul A, Yuen PST, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat Med.* 2009; 15(1):42–49. [PubMed: 19098906]
42. Papapetropoulos A, García-Cardena G, Dengler TJ, et al. Direct actions of angiopoietin-1 on human endothelium: evidence for network stabilization, cell survival, and interaction with other angiogenic growth factors. *Lab Invest.* 1999; 79(2):213–223. [PubMed: 10068209]
43. Thurston G, Suri C, Smith K, et al. Leakage-resistant blood vessels in mice transgenically overexpressing angiopoietin-1. *Science.* 1999; 286(5449):2511–2514. [PubMed: 10617467]
44. Thurston G, Rudge JS, Ioffe E, et al. Angiopoietin-1 protects the adult vasculature against plasma leakage. *Nat Med.* 2000; 6(4):460–463. [PubMed: 10742156]
45. Xu J, Qu J, Cao L, et al. Mesenchymal stem cell-based angiopoietin-1 gene therapy for acute lung injury induced by lipopolysaccharide in mice. *J Pathol.* 2008; 214(4):472–481. [PubMed: 18213733]
46. Lee JW, Fang X, Dolganov G, et al. Acute lung injury edema fluid decreases net fluid transport across human alveolar epithelial type II cells. *J Biol Chem.* 2007; 282(33):24109–24119. [PubMed: 17580309]
47. Fang X, Neyrinck AP, Matthay MA, Lee JW. Allogeneic human mesenchymal stem cells restore epithelial protein permeability in cultured human alveolar type II cells by secretion of angiopoietin-1. *J Biol Chem.* 2010; 285(34):26211–26222. [PubMed: 20554518]
48. Matthay MA, Wiener-Kronish JP. Intact epithelial barrier function is critical for the resolution of alveolar edema in humans. *Am Rev Respir Dis.* 1990; 142(6 Pt 1):1250–1257. [PubMed: 2252240]
49. Ware LB, Matthay MA. Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. *Am J Respir Crit Care Med.* 2001; 163(6):1376–1383. [PubMed: 11371404]
50. Dagenais A, Fréchette R, Yamagata Y, et al. Downregulation of ENaC activity and expression by TNF-alpha in alveolar epithelial cells. *Am J Physiol Lung Cell Mol Physiol.* 2004; 286(2):L301–311. [PubMed: 14514522]
51. Frank JA, Pittet J, Lee H, Godzich M, Matthay MA. High tidal volume ventilation induces NOS2 and impairs cAMP- dependent air space fluid clearance. *Am J Physiol Lung Cell Mol Physiol.* 2003; 284(5):L791–798. [PubMed: 12562562]
52. Frank J, Roux J, Kawakatsu H, et al. Transforming growth factor-beta1 decreases expression of the epithelial sodium channel alphaENaC and alveolar epithelial vectorial sodium and fluid transport via an ERK1/2-dependent mechanism. *J Biol Chem.* 2003; 278(45):43939–43950. [PubMed: 12930837]
53. Roux J, Kawakatsu H, Gartland B, et al. Interleukin-1beta decreases expression of the epithelial sodium channel alpha-subunit in alveolar epithelial cells via a p38 MAPK-dependent signaling pathway. *J Biol Chem.* 2005; 280(19):18579–18589. [PubMed: 15755725]
54. Folkesson HG, Matthay MA. Alveolar epithelial ion and fluid transport: recent progress. *Am J Respir Cell Mol Biol.* 2006; 35(1):10–19. [PubMed: 16514116]
55. Lee JW, Fang X, Gupta N, Serikov V, Matthay MA. Allogeneic human mesenchymal stem cells for treatment of E. coli endotoxin-induced acute lung injury in the ex vivo perfused human lung. *Proc Natl Acad Sci US A.* 2009; 106(38):16357–16362.
56. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science.* 1997; 276(5309):71–74. [PubMed: 9082988]

57. Krause DS, Theise ND, Collector MI, et al. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell*. 2001; 105(3):369–377. [PubMed: 11348593]
58. Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. *Proc Natl Acad Sci US A*. 1999; 96(19):10711–10716.
59. Kotton DN, Ma BY, Cardoso WV, et al. Bone marrow-derived cells as progenitors of lung alveolar epithelium. *Development*. 2001; 128(24):5181–5188. [PubMed: 11748153]
60. Liechty KW, MacKenzie TC, Shaaban AF, et al. Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep. *Nat Med*. 2000; 6(11):1282–1286. [PubMed: 11062543]
61. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood*. 2005; 105(4):1815–1822. [PubMed: 15494428]
62. Corcione A, Benvenuto F, Ferretti E, et al. Human mesenchymal stem cells modulate B-cell functions. *Blood*. 2006; 107(1):367–372. [PubMed: 16141348]
63. Glennie S, Soeiro I, Dyson PJ, Lam EW, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. *Blood*. 2005; 105(7):2821–2827. [PubMed: 15591115]
64. Spees JL, Olson SD, Whitney MJ, Prockop DJ. Mitochondrial transfer between cells can rescue aerobic respiration. *Proc Natl Acad Sci US A*. 2006; 103(5):1283–1288.
65. Plotnikov EY, Khryapenkova TG, Vasileva AK, et al. Cell-to-cell cross-talk between mesenchymal stem cells and cardiomyocytes in co-culture. *J Cell Mol Med*. 2008; 12(5A):1622–1631. [PubMed: 18088382]
66. Schorey JS, Bhatnagar S. Exosome function: from tumor immunology to pathogen biology. *Traffic*. 2008; 9(6):871–881. [PubMed: 18331451]
67. Deregibus MC, Cantaluppi V, Calogero R, et al. Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. *Blood*. 2007; 110(7):2440–2448. [PubMed: 17536014]
68. Ratajczak J, Miekus K, Kucia M, et al. Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. *Leukemia*. 2006; 20(5):847–856. [PubMed: 16453000]
69. Bruno S, Grange C, Deregibus MC, et al. Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. *J Am Soc Nephrol*. 2009; 20(5):1053–1067. [PubMed: 19389847]
70. Collino F, Deregibus MC, Bruno S, et al. Microvesicles derived from adult human bone marrow and tissue specific mesenchymal stem cells shuttle selected pattern of miRNAs. *PLoS ONE*. 2010; 5(7):e11803. [PubMed: 20668554]
71. Meirelles LDS, Fontes AM, Covas DT, Caplan AI. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. *Cytokine Growth Factor Rev*. 2009; 20(5–6):419–427. [PubMed: 19926330]
72. Feng J, Mantesso A, Sharpe PT. Perivascular cells as mesenchymal stem cells. *Expert Opin Biol Ther*. 2010; 10(10):1441–1451. [PubMed: 20836622]
73. Hare JM, Traverse JH, Henry TD, 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(24):2277–2286. [PubMed: 19958962]
74. Matthay MA, Thompson BT, Read EJ, et al. Therapeutic potential of mesenchymal stem cells for severe acute lung injury. *Chest*. 2010; 138(4):965–972. [PubMed: 20923800]

Figure 1. Beneficial effects of mesenchymal stem cells (MSCs) in acute lung injury. This figure depicts protein-rich edema fluid and inflammatory cells filling an injured alveolus after endothelial and epithelial injury. MSCs exert immunomodulatory effects on neutrophils, lymphocytes, and macrophages, assist in repair of the injured epithelial and endothelial barriers, improve alveolar fluid clearance, and secrete several molecules including the antibacterial peptide LL-37, angiopoietin-1 (ANG-1), and keratinocyte growth factor (KGF).

Table 1

MSCs in Models of Acute Lung Injury

Reference	Injury model	MSCs given	Beneficial effects	Increased	Decreased	Other
38	Intratracheal LPS in mice	Intratracheally, 4 hrs after LPS; Sac at 24 and 48 hrs	↓Lung edema ↓Lung hemorrhage ↓BAL protein ↓Mortality	IL-10	MIP-2, TNF- α	No significant engraftment
37	Intraperitoneal LPS in mice	Intravenously, 1 hour after LPS; Sac at 6, 24, 48 hrs and 14 days	↓Lung PMNs ↓Lung edema		IFN- γ , IL-1 β , MIP-1 α , KC	No significant engraftment
40	Cecal ligation and puncture in mice	Intravenously 6 hours after CLP; Sac at 28 hrs	↓BAL cell counts ↓BAL albumin ↓Lung Edema and inflammation ↓Kidney injury ↓Mortality		IL-6, IL-1 β , IL-10, KC, JE, CCL5	Microarray analysis: downregulation of inflammatory genetic pathways; Enhanced bacterial clearance
41	Cecal ligation and puncture in mice	Intravenously 24 hours before or 1 hour after CLP; Sac at 1 and 4 days	↓Kidney injury ↓Transaminases ↓Splenic apoptosis ↓Mortality ↓Tissue myelo-peroxidase	IL-10, PGE2	TNF- α and IL-6	In vitro MSCs reprogram monocytes/macrophages to secrete IL-10
39	Intratracheal E-coli in mice	Intratracheally 4 hours after E-coli; Sac at 18 hrs	↓BAL neutrophils ↓BAL protein		MIP-2	MSC-produced LL-37 decreases bacterial growth in vitro and BAL bacterial growth in vivo