

Adult Mesenchymal Stem Cells: An Innovative Therapeutic for Lung Diseases

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Author: **Tracey L. Bonfield**

Specialty: Immunology, Stem Cells, Pulmonology, Pathology

Institution: Division of Pediatric Pulmonology, Case Western Reserve University School of Medicine

Address: 10900 Euclid Avenue, Cleveland, Ohio, 44106, United States

Author: **Arnold I. Caplan**

Specialty: Stem Cells, Biology

Institution: The Skeletal Research Center, Case Western Reserve University School of Medicine

Address: Cleveland, Ohio, 44106, United States

Abstract: Adult human mesenchymal stem cells (hMSCs) are the focus of a number of clinical applications. The advantage of hMSCs is that they are immuno-modulatory and versatile due to their secreted bioactive molecules that are anti-inflammatory and regenerative. These cells have the potential to orchestrate reparative processes in diseased or injured tissues. Much of the diversity and uniqueness of hMSCs is defined by their response to the milieu of injured tissue. hMSCs are sensitive to their site-specific microenvironment, and it is anticipated that they will deliver the bioactive agents in a site-specific manner quite different from the way pharmaceutical drugs work. This review highlights current concepts of such functions with a focus on the clinical utility of hMSCs in the treatment of lung diseases.

Mesenchymal Stem Cells

In the late 1980's, Arnold Caplan and colleagues developed and patented the technology to isolate adult hMSCs from human bone marrow and to mitotically expand these cells in culture while preserving their multi-potency (Caplan et al., 2001; Koc et al., 1999; Lennon et al., 2006). Adult human MSCs are capable of differentiating into a number of phenotypes, which include cells capable of fabricating bone, cartilage, muscle, marrow, tendon/ligament, adipocytes, and connective tissue(**Figure 1**). Furthermore, hMSCs have been shown to produce large quantities of bioactive factors which provide molecular cueing for regenerative pathways as well as affecting the status of responding cells intrinsic in the tissue (Caplan et al., 2006; Haynesworth et al., 1996). Recently, as an added advantage, it has been shown that hMSCs are well tolerated across species, and it was documented these cells they may be useful in xenographic transplantation and thereby provide an alternative source of cells in pre-clinical models (Mimeault et al., 2007; Caplan et al., 2001). The apparent immune-privileged property of adult hMSCs also provides the opportunity to use animal models to begin to define mechanisms of hMSC function and efficacy.

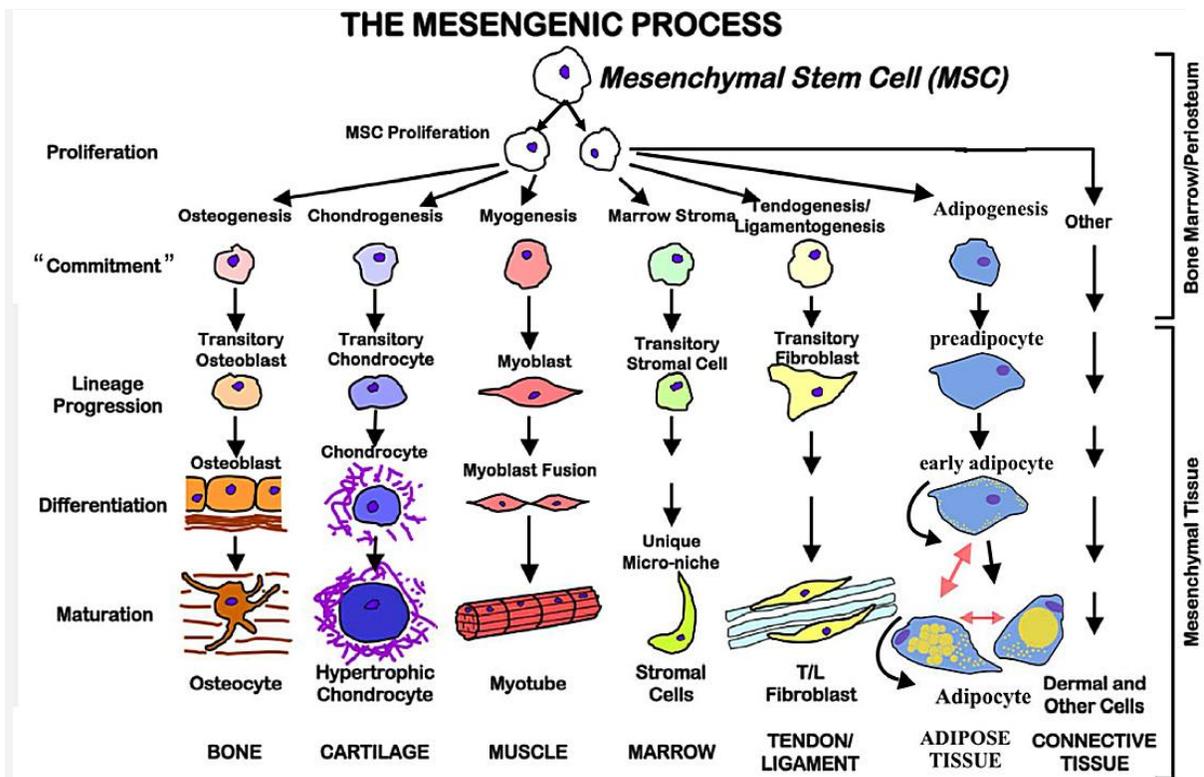


Figure 1. Mesenchymal stem cell differentiation. Mesenchymal stem cells (MSCs) can be cultured *in vitro* to generate a variety of differentiated cells and demonstrate their multi-potent capacity and differentiation plasticity. The end-stage cell type is dependent on the culture conditions, media, and supplements.

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hMSCs are non-hematopoietic, multi-potent progenitor cells that have the capacity to generate bone marrow stromal cells as well as adipocytes, chondrocytes, and osteocytes in appropriate tissue and other organ sites (Abdallah et al., 2007). Currently, there is no single unique marker for hMSCs, although the absence of CD34 and CD45 and the presence of SH2, SH3, SH4, Stro-1, and others are used to identify hMSCs (Dominici et al., 2006). *In vitro* assays for the development of bone, cartilage (Lennon et al., 2000), marrow stroma, and fat (Banas et al., 2007) have been used to define hMSCs, but none of these endpoints of activity can define *in vivo* trophic responses. In addition, the secreted products of hMSCs have the capacity to change the milieu of both immune and regenerative activities. The medium conditioned by activated hMSCs has been shown to be immunosuppressive in mixed lymphocyte ELISA-spot assays for T-cell activity (Caplan et al., 2006) and to influence the differentiation of neural stem cells (Caplan et al., 2007).

Although MSCs can differentiate into various phenotypes of mature cells, their intrinsic capacity to secrete cytokines and growth factors at sites of tissue injury and inflammation contribute significantly to their therapeutic capacity. We assume that the production of these trophic mediators is defined by their *in vivo* location, niche, and severity of injury (Abdallah et al., 2007; Caplan et al., 2006). MSCs are reservoirs for the production of cytokines, chemokines, and extracellular matrix which have the ability to support stem cell survival and proliferation (Abdallah et al., 2007). More importantly, the cytokines and chemokines produced by hMSCs

have the ability to influence multiple immune effector functions including cell development, maturation, and allo-reactive T-cell responses (Chamberlain et al., 2007). Since hMSCs can be expanded *ex vivo* and because *in vivo* models have shown infusion safety, clinical studies have begun to investigate the use of hMSCs as multifunctional cellular therapeutic agents. Allogeneic hMSCs can suppress graft versus host disease (GvHD) and can have profound regenerative capacity in the case of stroke, infarct, spinal cord injury, meniscus regeneration, tendonitis, acute renal failure and, recently, heart disease (www.osiris.org; Amsalem et al., 2007; Christopheit et al., 2008; Abdel Aziz et al., 2007; Bao et al., 2008). Systemic infusion of allo-hMSCs into pediatric GvHD patients resulted in complete remission (Subbanna et al., 2007). In this study, one patient relapsed and required a second round of multiple infusions of hMSCs. Although a single observation, it raises questions with regard to the dose of hMSCs, route of administration, and the possibility that the clinical outcomes may require more than one round of multiple infusions in certain patients. Furthermore, the requirements for additional infusions may also be related to the potency of the hMSC preparation. hMSCs produce large quantities of bioactive factors which provide molecular signatures for the pathway and activity status of the responding cells (Lennon et al., 1995). These bioactive factors are: (1) immunosuppressive for T-cells and other immune cells, (2) anti-scarring, (3) angiogenic, (4) anti-apoptotic, and (5) regenerative (for host tissue). Therefore, not only can hMSCs function in inducing host regenerative capacity, but may also actively participate in altering acute and chronic inflammatory responses (Caplan et al., 2009). Since hMSCs are sensitive to their site-specific microenvironment, it is anticipated that they will deliver the bioactive agents in a site-specific manner quite different from pharmaceutical drugs currently on the market.

MSCs: The Other Facts

In contrast to the idea that the key function of hMSCs is to supply replacement parts mesenchymal tissue are the observations that human marrow-derived MSCs can be activated to secrete cytokines/growth factors which inhibit an *in vitro* mixed lymphocyte assay (Abboud et al., 1987; Maitra et al., 2004). These observations suggested that hMSCs could be used therapeutically as allogeneic, “universal cells.” To support this suggestion, it has been further documented that culture-expanded hMSCs do not have MHC class I cell surface markers, but rather only MHC class II and no co-stimulator molecules (Dominici et al., 2006). Thus, hMSCs cannot be antigen-presenting cells and should be invisible to the host’s immune system. It is important to note that in all of the clinical usages of human adult marrow-derived, culture-expanded MSCs, whether autologous or allogeneic, no adverse events have been recorded (Abdallah et al., 2007; Le Blanc et al., 2007). This establishes that the procedures for isolation and culture expansion are safe and that in certain clinical applications, there has been a benefit from the intravenous delivery of hMSCs.

hMSCs can be of great value by virtue of their ability to differentiate into distinctive and specialized cells and their secretion of site-specific proteins. Defining the mechanisms of MSC therapeutic efficacy may require elaborate technology associated with delivery, imaging, and targeting, which has the potential of identifying appropriate delivery mechanisms and the ability to localize hMSCs (Caplan et al., 2007). The use of hMSCs in interstitial pulmonary fibrosis, cardiovascular disease, and neurological disorders is currently being explored and was based upon not only the ability of hMSCs to be site-specific producers of trophic factors, but also upon their ability to induce host tissue trophic factors which may also contribute to the inflammatory milieu (Dai et al., 2005; Stripp et al., 2006). It remains to be determined if hMSCs home to the site of inflammation and/or if the MSCs’ destination is a central or systemic site

such as the lymph system. Caplan and his colleagues have recently described a cell targeting technology for such site-directed therapeutics (Dennis et al., 2004). The issue is where to target the therapeutic cell for the most impact and largest clinical response. Targeting will depend upon the clinical entity in question and the stage and severity of the disease. The studies outlined in this review focus on lung airway inflammation and remodeling and the therapeutic potential of hMSCs.

Clinical Utility of MSC Therapy

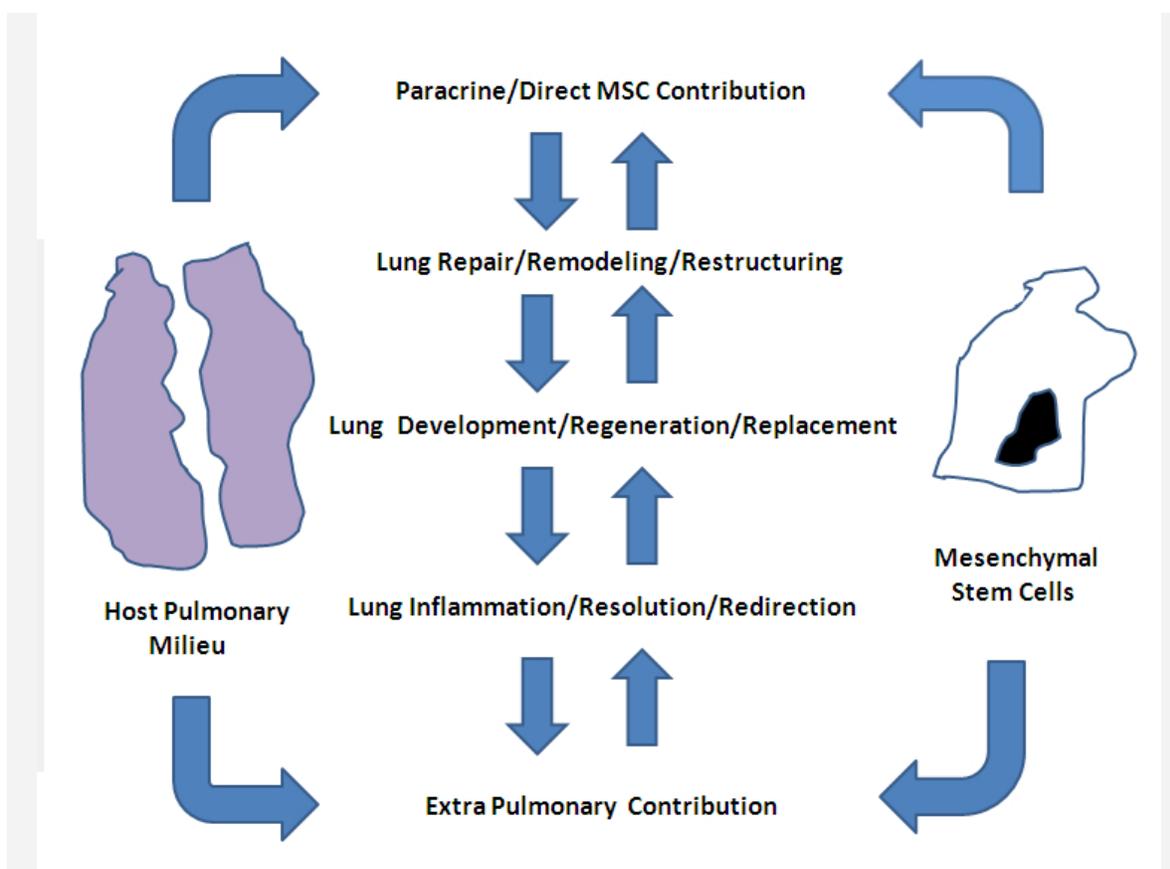


Figure 2. Complexity of human mesenchymal stem cells in in vivo reactions in the lung. Application of hMSCs in lung disease is a complex process with tissue communication, secretion of paracrine factors, and disease-specific outcomes. This figure shows the complex interaction between infused hMSCs and the tissue milieu. There is potentially an interchange of communication at every step as the stem cells travel throughout the host. The basic science challenge in hMSC therapy is focusing on understanding the mechanisms of hMSC function and monitoring appropriate translational outcomes in the context of tissue response.

As described above, hMSCs have the capacity to uniquely impact the targeted tissue. In diseases involving tissue damage, the expectation is that the systemically delivered hMSCs would repair the damaged tissue or, alternatively, stimulate the host tissue to regenerate itself. This active involvement between the hMSCs and the tissue milieu is due to the interactive nature of the hMSCs with the surrounding environment. In conditions with a large amount of

fibrotic disease, the hMSC activity would involve reversal of extracellular matrix deposition and collagen synthesis. The hMSC activity is involved with inducing a direct response to remove the fibrotic tissue or a host response to repair itself. In scenarios of overt inflammation, the goal would be to attenuate the inflammatory response without inducing susceptibility to infection or tissue rejection. **Figure 2** shows the flexibility of the hMSC within the given tissue scenario and the obvious potential of hMSCs to be directors of host response based on the products they produce and the response of the host tissue. The regenerative processes are probably dependent on both the properties of the hMSC and of the host tissue. The impact of lung disease and the versatility of the hMSCs can be divided into three different focal points: lung development, lung repair, and anti-inflammatory therapeutics (Iyer et al., 2008; Iyer et al., 2009). The issue is the tissue milieu, which has the capacity to set the stage for the host and donor hMSC cellular interaction and exchange. Defining the contribution of the donor versus the host response to this exchange is difficult to decipher since, in most cases, the infusion is allogeneic. On the other hand xenographic infusion of hMSCs into murine or rat models introduces the potential for cross-species response, even with the concept that the hMSCs are immune-privileged due to the lack of MHC class I. In this regard, xenographic administration also allows the elucidation of host versus donor response to the disease in the context of the administration of the hMSCs.

MSCs in Lung Disease

Stem cells are unique cells, which have the capacity for self-renewal, differentiation, and ability to conform to a specific identity in response to host cells, cytokines, and extracellular components (Chamberlain et al., 2007). This environment may accommodate the cells indefinitely and control their self-renewal and progeny production *in vivo* or refine their ability to induce host reparative processes. The question remains as to whether the beneficial observations of using hMSCs in diseases are a result of repair and regeneration or simply due to the attenuation of the inflammatory response inherent in cell therapy. **Table 1** summarizes several pulmonary diseases in which murine models have been utilized to study the therapeutic implication of adult human MSCs *in vivo*.

Table 1. Models of Lung Disease, Mesenchymal <u>Stem Cell Therapy</u>, and Clinical Application.		
Lung Disease	Animal Model	References
Idiopathic Pulmonary Fibrosis (IPF)	Bleomycin-induced pulmonary fibrosis	Gharaee-Kermani et al., 2007
Acute Lung Injury (ALI)/Adult Respiratory Distress Syndrome (ARDS)	<u>Endotoxin</u> -induced <u>sepsis</u> and lung injury	Lee et al., 2009; Matthay et al., 2008; Johnson et al., 2010
Chronic Obstructive Pulmonary Disease (COPD)	Smoking-induced <u>emphysema</u>	Braun et al., 2006; Allison et al., 2009
Idiopathic <u>Pulmonary Hypertension</u> (IPH)	Endothelial damage	Weiss et al., 2008; Weiss et al., 2009
Neonatal Pulmonary Insufficiency	Alveolar and epithelial immaturity	van Haften et al., 2009

(BPD:Bronchopulmonary Dysplasia)		
Radiation Induced-Pulmonary Injury	Radiation-induced alveolar damage and cell death	Kursova et al., 2009
Cystic Fibrosis (CF)	<u>Knockout</u> models of <u>CFTR</u>	Weiss et al., 2008; Sueblinvong et al., 2009
<u>Asthma</u>	Ovalbumin models of sensitization and challenge	Weiss et al., 2008

Idiopathic pulmonary fibrosis (IPF) is an interstitial inflammatory disease with unknown etiology characterized by scarring and fibrosis of the lungs that ultimately result in terminal pulmonary insufficiency. In studies using the bleomycin model, which mimics much of the pathogenic morphology of IPF, hMSC administration immediately after bleomycin exposure resulted in a significant reduction in inflammation and collagen deposition (Neuringer et al.; 2006, Ortiz et al., 2003). The rates of cellular engraftment and differentiation in these studies were very small and, according to the authors, not detectable. The implication highlighted in these studies is that the mechanism of the hMSC effect and improvement in bleomycin-induced inflammation was due to the paracrine secretion of growth factors and cytokines that ultimately stimulate host repair and regeneration. Interleukin-1 receptor antagonist has been implicated as a potential mediator of this effect potentially due to its ability to block IL-1 β pro-inflammation signaling (Ortiz et al., 2007). An alternative mechanism suggests that murine hMSCs home to lung in response to injury, ultimately adopting an epithelium-like phenotype, reducing inflammation and collagen deposition in lung tissue of mice challenged with bleomycin (Ortiz et al., 2003). The question remains as to whether the mechanism of decreased inflammation and lung pathology is through paracrine/autocrine effects of the MSCs and/or host tissue or through an active reparative process. The issue may be related to the source of MSCs, the end points of analysis, and model differences.

Acute lung injury (ALI) is a significant source of morbidity and mortality of patients in intensive care settings. ALI is defined by the acute onset of pulmonary infiltrates with severe hypoxemia (Johnson et al., 2010; Rubenfeld et al., 2005). The pathogenesis of ALI has been associated with injury to vascular endothelium and alveolar epithelium as well as sensitivity to endotoxin. In a mouse model of ALI, *Escherichia coli* endotoxin was instilled into the distal airspaces of the lung followed by direct intrapulmonary administration of murine MSCs several hours later. Mice treated with murine MSCs had decreased extra-vascular lung edema, alveolar capillary permeability, and mortality (Lee et al., 2009; Matthay et al., 2008). The pro-inflammatory response to the endotoxin was decreased, while the resolution response and anti-inflammatory cytokines were enhanced. In a novel ALI model using *ex vivo* perfused human lung preparations, allogeneic human MSCs improved alveolar fluid clearance following endotoxin-induced lung injury. However, several issues remain to be investigated prior to translation of allogeneic or autologous hMSC research findings into clinical applications (Sueblinvong et al., 2009; Matthay et al., 1999). These *ex vivo* and murine studies implicate the use of adult human MSCs in the treatment of ALI and its closely associated adult respiratory distress syndrome (ARDS).

Radiation-induced lung injury is one of the most severe complications of post-radiological treatment for cancer (Kursova et al., 2009). The injury increases susceptibility to lung infection, pneumonitis, and pulmonary fibrosis. Patients with these complications ultimately have

respiratory insufficiency which adversely affects the quality of life. In the murine model of radiation-induced lung injury, administration of murine MSCs significantly decreased mortality and improved pulmonary function. Patients with radiation-induced lung fibrosis and pneumonitis who were given adult human MSCs showed pulmonary stabilization without exacerbation of malignancy. This is important since there have been some concerns regarding the transformation potential of hMSCs *in vivo* and whether they might enhance tumorigenesis. Bronchopulmonary dysplasia (BPD) and emphysema are characterized by arrested alveolar development or loss of alveoli as an outcome of immature lungs of premature babies. This ultimately results in neonatal hyperoxia (van Haaften et al., 2009; Nogee et al., 2004). In a rat model of BPD, administration of autologous MSCs resulted in the prevention of BPD, allowing for maturation of the lung with alveolar and vascular growth. Whether this was due to the tissue response to the autologous rat MSCs or a direct effect of the MSCs was not defined. However, the mechanisms by which the rat MSCs induce differentiation of the vascular and alveolar compartment correlated with enhanced expression of surfactant protein C and the presence of lamellar bodies, both of which being essential for final maturation and function of the lung. Similarly designed studies are projected to apply MSC therapy to patients with arrested lung and vascular growth in chronic lung diseases associated with prematurity.

Chronic obstructive pulmonary disease (COPD) is a lung disease which includes both chronic bronchitis and emphysema (Shigemura et al., 2006; Senior et al., 1998). COPD is one of the leading causes of death and disability in the United States and which results predominantly from cigarette smoking. Pilot studies using allogeneic MSCs were initiated for the treatment of steroid-resistant COPD; however, the outcomes were disappointing (Allison et al., 2009). The trial concluded early not due to adverse events, but due to the inability to reach the defined definition of response which was a sustained increase in pulmonary function measured by forced-expiratory volume (FEV1). In these initial pilot studies, even the placebo group had a higher than expected improvement of FEV1. The lack of response to the MSCs might have been related to dosage, route of administration, the number of infusions, the stage of patient with COPD, and the potency of the hMSC preparation (Allison et al., 2009; Caplan et al., 2009). New trials are being designed to better capture patient response and accurate recording.

Cystic fibrosis (CF) is a common hereditary disease which affects mucous clearance, infection resolution, and nutrition (Nichols et al., 2008). The genetic mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) impact the entire body, causing progressive decrease in quality of life and premature death. Although CF is an endocrine disorder, the main cause of morbidity and mortality is pulmonary insufficiency, deficient mucociliary clearance, and chronic infection with *Pseudomonas aeruginosa*. Published studies implicated the potential of using bone marrow transplantation as a method of attenuating the overt inflammatory response in the CF lung (Bruscia et al., 2009; Weiss et al., 2006). We have shown that bone marrow transplantation into CFTR-null animals significantly improved the response of mice chronically infected with *Pseudomonas aeruginosa*, ultimately improving clinical scores and lung pathology (unpublished observations). Further, in preliminary unpublished observations, administration of adult hMSCs improved outcome measures and decreased inflammation in the same chronic model of infection and inflammation. These studies implicate the potential of cell-based therapy in CF.

Asthma is one of the most common chronic inflammatory diseases associated with airway reactivity and inflammation that result in lung injury (Boyton et al., 2004; Masoli et al., 2004). Published observations have shown that administration of murine MSCs in the ovalbumin model of acute asthma significantly decreased airway hyper-responsiveness as well as the

number of eosinophils in bronchoalveolar lavage fluid (Weiss et al., 2008). The murine MSCs also influenced the direction of T-cell response shifting the trend of the T-cell phenotype away from T_H2 cytokines. This effect was not dependent on an autologous source of the bone marrow-derived murine MSCs. In our own murine models of both acute and chronic asthma, we have found that administration of human MSCs resulted in a decrease in inflammation, epithelial hyperplasia, and extracellular matrix deposition (manuscript in submission). The xenographic administration of hMSCs in the murine model did not result in any detectable adverse side effects, again emphasizing the immuno-privileged and immune-modulatory nature of MSCs.

Translation of Mesenchymal Stem Cell Therapy to Clinical Applications

The issues related to translating MSCs from animal models into the clinical setting are highlighted in **Table 2**. Standardization of methods to define MSC classification, isolation, efficacy, and potency are required for consistency in clinical trials (da Silva et al., 2008; Dominici et al., 2006). It is known that different preparations and, indeed, different donors have different biological impacts based upon the *in vivo* model and the potency of the preparations. A standardization of protocols and classifications needs to be accomplished prior to understanding both the potency and efficacy of MSC preparations. The heterogeneity in hMSC effectiveness is also clouded by the potential variation in the tissue sources for MSCs, which can also impact efficacy and potency *in vivo*. Currently there are no *in vivo* efficacy models to measure MSC “bioactivity.” Our group has recently developed a potency assay using a murine model of lung inflammation which can be attenuated by MSC therapy (manuscript in submission). In this model, MSCs given after the onset of inflammation had a significant ability to reduce pulmonary neutrophil and eosinophil recruitment. Further, similar to the studies described above, introduction of hMSCs resulted in a shifting of cytokine profiles away from a T_H2 phenotype (Weiss et al., 2008). Future directions will focus on correlating the success of MSC therapy in the murine asthma model to dosing and route of administration (Abdallah et al., 2007; Deans et al., 2000). Since mechanistically it is unknown whether the hMSCs act directly or through the orchestration of the immune response, focused attention must be made on the endpoints and markers of evaluation. This will also impact the success of the studies since different models have, at their respective center, different pathological processes. Finally, developments in imaging technology capable of tracking hMSCs post-administration will help in delineating function and site of action in the context of the lung disease.

Table 2. Issues in Understanding Mesenchymal Stem Cell Mechanism of Action <i>In Vivo</i>.		
Issue	Differences	Approaches
Classification	Define phenotypes and functions.	Negative selection, <u>flow cytometry</u> , <i>in vivo</i> modeling.
Efficacy	Define the success of hMSCs within a study.	Reproducible effects <i>in vitro</i> , <i>in vivo</i> , and clinically.
Potency	How much is enough for hMSC activity?	Comparison of dosage to endpoint of response.
Mode of administration	Does systemic versus localized administration	Direct comparison of localized versus systemic administration in

	make a difference?	the same model system with the same preparation.
Dosage	How many and how many times do hMSCs need to be administered for effectiveness?	Achieving enhanced efficacy and potency.
Source of MSCs	Fat tissue, amniotic, bone marrow.	Different sources may have different efficacy, potency, and function.
Endpoint of analysis	Inflammation, reparative, regenerative.	Define success of output related to disease of interest.
MSC tracking	Transformation potential, area of impact, local or distance orchestration through the lymphatics	Issues related to tracking for host response and changes in hMSC phenotype.

Summary

Mesenchymal stem cells exhibit extensive diversity in differentiation, production of trophic mediators, and interaction with the host environment. Clinical studies are ongoing in a variety of inflammatory diseases to determine if the unique functions of hMSCs may have a therapeutic impact on disease attenuation and potential cure. The versatility of MSCs in terms of interaction with inflammatory or fibrotic processes make the adult-derived cells an attractive potential therapeutic for the treatment of diseases such as asthma, cystic fibrosis, neonatal dysplasia, acute lung injury, and chronic obstructive pulmonary disease. The issues are that the technology is in its infancy, with ongoing studies to define the unique phenotypic identification of MSCs, their efficacy *in vivo*, dosage, route of administration, and the duration of their potency. Applications of MSCs in chronic lung disease should be embraced at both the bench and bedside and carried into the clinic to provide new avenues for patient care.

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