PROSPECTS
Journal of Cellular Biochemistry 115:1023–1032 (2014)

Journal of Cellular Biochemistry

Mesenchymal Stem Cell Trials for Pulmonary Diseases

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ABSTRACT

All adult tissues, including the lung, have some capacity to self-repair or regenerate through the replication and differentiation of stem cells resident within these organs. While lung resident stem cells are an obvious candidate cell therapy for lung diseases, limitations exist regarding our knowledge of the biology of these cells. In contrast, there is considerable interest in the therapeutic potential of exogenous cells, particularly mesenchymal stem/stromal cells (MSCs), for lung diseases. Bone marrow derived-MSCs are the most studied cell therapy for these diseases. Preclinical studies demonstrate promising results using MSCs for diverse lung disorders, including emphysema, bronchopulmonary dysplasia, fibrosis, and acute respiratory distress syndrome. This mini-review will summarize ongoing clinical trials using MSCs in lung diseases, critically examine the data supporting their use for this purpose, and discuss the next steps in the translational pathway for MSC therapy of lung diseases. J. Cell. Biochem. 115: 1023–1032, 2014.

KEY WORDS: EMPHYSEMA; FIBROSIS; MESENCHYMAL STEM CELLS; ACUTE RESPIRATORY DISTRESS SYNDROME

All adult tissues, including the lung, have some capacity to self-repair or regenerate through the replication and differentiation of stem cells resident within these organs [da Silva Meirelles et al., 2006]. However, our knowledge regarding lung resident stem cells remains relatively incomplete, and considerable hurdles remain before clinical testing of lung stem cells for pulmonary diseases can be contemplated. In contrast, there is considerable interest in the therapeutic potential of exogenous cells, particularly mesenchymal stem/stromal cells (MSCs), for lung diseases. These cells are normally resident in the bone marrow, which contains two well distinct subpopulations of cells: hematopoietic stem cells (HSCs), supporting blood cell formation, and MSCs, which act in the marrow to facilitate maturation of HSCs but which are capable themselves of differentiating into other cell types.

Bone marrow derived-MSCs are the most studied cell therapy for lung diseases. These cells do not have a single defined characteristic, but are identified according to the following consensus criteria [Dominici et al., 2006]: adherence to plastic under standard culture conditions; expression of CD105, CD73, and CD90 and lack of surface expression of CD45, CD34, CD14, CD11b, CD79, CD19, and HLA-DR; and ability to differentiate into adipocytes, chondrocytes, and osteocytes in vitro. Different populations of MSCs have also been isolated from other adult tissues—brain, spleen, liver, kidney, lung, bone marrow, muscle, thymus, pancreas, fat—and fetal tissues [da Silva Meirelles et al., 2006; Nora et al., 2012; Li et al., 2013]. In fact, distinct anatomical populations of MSCs exhibit different immunophenotypes, secreted cytokine profiles, and proteome analyses [Ostanin et al., 2011].

MAIN MECHANISMS OF ACTION

MSCs have been suggested to be the best eligible candidates for allogeneic transplantation due to their immunomodulatory properties and their ability to secrete trophic factors. In this regard, MSCs home specifically to injured tissue and exert their immunomodulatory activity with the secretion of angiogenic (e.g., vascular endothelial growth factor) [Guan et al., 2013], anti-apoptotic (Bcl-2) [Zhen et al., 2008], and anti-inflammatory factors (e.g., interferon-γ,

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Manuscript Received: 2 February 2014; Manuscript Accepted: 6 February 2014
Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 11 February 2014
DOI 10.1002/jcb.24783 • © 2014 Wiley Periodicals, Inc.

interleukin-10, vascular endothelial growth factor, hepatocyte growth factor) [Rojas et al., 2005; Shigemura et al., 2006; Gupta et al., 2007; Nemeth et al., 2009; Guan et al., 2013], stimulating angiogenesis, promoting a secure environment for host cell recovery, and repairing and regenerating the injured tissue.

Exogenously administered MSCs modulate the function of host cells within the injury and reparative microenvironment, both by cell contact-dependent and paracrine mechanisms involving secretion of specific mediators and by the transfer of cellular materials such as proteins, nucleic acids, and cell organelles (including mitochondria) to injured host cells via microvesicles [Islam et al., 2012; Zhu et al., 2014]. Based on the foregoing, preclinical studies reported promising results using MSCs for lung disorders, including emphysema [Zhen et al., 2010; Guan et al., 2013], bronchopulmonary dysplasia [Chang et al., 2009; van Haaften et al., 2009; Chang et al., 2011], fibrosis [Cargnoni et al., 2009; Moodley et al., 2009; Lee et al., 2010], and acute respiratory distress syndrome [Gupta et al., 2007; Nemeth et al., 2009; Mei et al., 2010].

This mini-review aims to summarize ongoing clinical trials using MSCs in lung diseases, addressing the safety and feasibility of their use and the main findings that have already been obtained.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Chronic obstructive pulmonary disease (COPD) is the fourth most common disease in the world. It is characterized by persistent airflow limitation that is usually progressive and driven by an enhanced chronic inflammatory response in the airways and lung tissue in response to noxious particles or gases, especially cigarette smoke exposure, as well as genetic predisposition (α1-antitrypsin deficiency) [Global Strategy for the Diagnosis, Management and Prevention of COPD, 2014]. Cigarette smoking is the commonest preventable cause of COPD. This chronic inflammation causes structural changes and narrowing of the small airways. Destruction of the lung parenchyma, also by the inflammatory process, leads to the loss of the alveolar attachments to the small airways and decreases elastic recoil, which diminishes the ability of the airways to remain open, resulting in their collapse during expiration [Global Strategy for the Diagnosis, Management and Prevention of COPD, 2014]. COPD is characterized by a specific pattern of inflammation involving an increased number of CD8+ cytotoxic lymphocytes, neutrophils and macrophages, which release inflammatory mediators and enzymes and interact with structural cells of the airways (epithelial cells and fibroblasts), lung parenchyma (alveolar epithelial cells), and pulmonary vasculature (endothelial cells), leading to inflammation and destruction of the pulmonary tissue [Global Strategy for the Diagnosis, Management and Prevention of COPD, 2014].

In severe cases, COPD is considered a systemic disorder triggered by the primary pulmonary injury. Moreover, hypoxic vasoconstriction results in intimal hyperplasia and smooth muscle hypertrophy/hyperplasia, which in severe cases may lead to frank pulmonary arterial hypertension (PAH). The enzymatic destruction of the lung parenchyma leads to the loss of the pulmonary capillary bed in emphysema, also contributing to PAH. Both mechanisms result in right ventricular hypertrophy and eventual progression to cardiac failure. Furthermore, the inflammatory mediators present in the blood stream may contribute to skeletal muscle wasting and cachexia, and may trigger or worsen comorbidities.

Current therapies aim to control symptoms, limit inflammation, and enhance functional capacity in patients with COPD. However, no available therapy has been able to reconstitute the alveolar architecture or halt the fibrogenic process. MSCs may offer therapeutic potential for patients with COPD. In several preclinical studies (Fig. 1), MSC treatment has been demonstrated to attenuate inflammation by decreasing levels of inflammatory mediators, such as IL-1β, TNF-α, IL-8, as well as decrease apoptosis [Zhen et al., 2010; Huh et al., 2011], improve parenchymal repair (increased levels of keratinocyte growth factor, hepatocyte growth factor and epidermal growth factor), and increase lung perfusion [Shigemura et al., 2006; Huh et al., 2011; Guan et al., 2013]. Based on these preclinical findings, several groups are investigating the therapeutic potential of MSC therapy in COPD patients. To date, two early-phase clinical trials have finished and six are ongoing in this patient population.

The first safety trial in COPD registered in ClinicalTrials.gov (NCT01110252) using bone marrow mononuclear cells (BMMCs), which encompasses the whole fraction of HSCs and MSCs from bone marrow, was carried out in Brazil. On 12-month follow-up, four patients/volunteers with advanced COPD (stage IV dyspnea) exhibited no adverse effects of BMMC therapy, and experienced a significant improvement in the quality of life consistent with a more clinical stable condition [Ribeiro-Paes et al., 2011]. Since then, additional trials have commenced in several countries to further examine the safety and possible efficacy of MSC therapy for COPD (Table I). The only such trial that has been published to date was carried out in the United States (NCT00683722), using intravenous allogeneic MSCs (PROCHYMAL®; Osiris Therapeutics Inc). Sixty-two patients were randomized to double-blinded intravenous infusions of either allogeneic MSCs or vehicle control. Patients received four monthly infusions (100×10^6 cells/infusion) and were subsequently followed for 2 years after the first infusion [Weiss et al., 2013]. Endpoints included comprehensive safety evaluation, pulmonary function testing (PFT), and quality-of-life indicators including questionnaires, 6-min walk test (6MWT), and assessments of systemic inflammation. This trial demonstrated that use of MSCs in COPD patients may be considered safe, as there were no infusion reactions and no deaths or serious adverse events deemed related to MSC administration. However, no significant differences were observed in the overall number of adverse events, frequency of COPD exacerbations, or severity of disease as measured by PFTs and quality-of-life indicators in patients treated with MSCs. Of interest, the subgroup of patients who had elevated circulating C-reactive protein levels at baseline demonstrated a significant decrease following MSC infusion [Weiss et al., 2013].

In Brazil, a phase I, non-randomized, open-label study is currently recruiting patients diagnosed with severe heterogeneous emphysema to evaluate the safety of one-way endobronchial valves combined with bone-marrow MSCs (NCT01872624). This study will have a 4-month follow-up period to test the safety of the procedure, as assessed by evaluations of quality of life, pulmonary function, and inflammatory status (blood samples for C-reactive

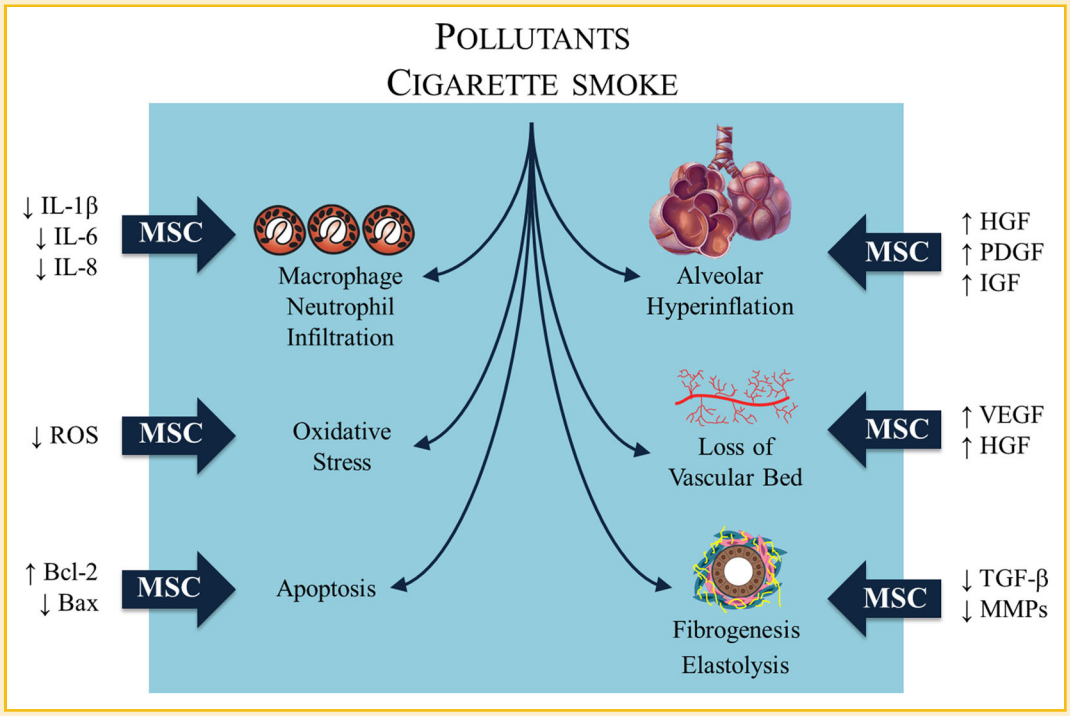

Fig. 1. Potential mechanisms of action of MSCs in COPD. ROS: reactive oxygen species; IL: interleukin; Bcl-2: anti-apoptotic protein; Bax (or Bcl-2-associated X protein): pro-apoptotic protein; HGF: hepatocyte-derived growth factor; PDGF: platelet-derived growth factor; IGF: insulin-like growth factor; VEGF: vascular endothelial growth factor; TGF-β: transforming growth factor-β; MMPs: matrix metalloproteinases.

protein and erythrocyte sedimentation rate, complete blood count in peripheral blood). In the Netherlands, a phase I, non-randomized, non-blinded, prospective study to test the safety and feasibility of administration of bone-marrow MSCs before and after lung volume reduction surgery (LVRS) for severe pulmonary COPD has been concluded (NCT01306513). In this trial, 10 COPD patients (GOLD III) received two intravenous administrations of autologous bone-marrow MSCs performed after one-sided LVRS and prior to a second LVRS (3 and 4 weeks before) in the contralateral lung. These investigators expect to observe differences in days between postsurgical transpleural air leak of the lung in each patient after the first (before bone-marrow MSC administration) and second LVRS (3 weeks after the last intravenous bone-marrow MSC dose), as well as different histological responses in resected lung tissue (measured by immunohistochemistry of markers of inflammation, fibrosis, and repair), but so far, no data have been published.

In Russia, a phase I/II, randomized, placebo-controlled study has been designed to evaluate the safety and efficacy of intravenous infusions of allogeneic bone-marrow MSCs. In this study, 2×10^8 MSCs (hypoxic-preconditioned in 1% oxygen) will be administered to patients with severe COPD (NCT01849159) every 2 months over a 1-year period. This ongoing study will examine the efficacy and safety of MSC transplantation, using three different efficacy endpoints (lung tissue density measured by CT-densitometry, pulmonary function, and diffusion capacity [DLCO]) assessed at 6, 12, and 24 months. This study has an estimated completion date of late 2014, but so far, it has not been opened for participant recruitment.

TABLE I. Clinical Trials of Mesenchymal Stem Cell Therapy in COPD

Location	Patients	Cell type	Dose	Frequency	Delivery	Follow-up	Status	ClinicalTrials.gov
Brazil	4	BMDMC	1×10^8/ml	Single dose	Intravenous	12 months	Completed	NCT01110252
USA	62	BM-MSC	1×10^8	Four monthly	Intravenous	2 years	Completed	NCT00683722
Brazil	10	BM-MSC	*	Single dose	Endobronchial	4 months	Recruiting	NCT01872624
Netherlands	10	BM-MSC	*	Twice weekly	Intravenous	8 weeks	Completed	NCT01306513
Russia	30	BM-MSC	2×10^8	Every 2 months for 1 year	Intravenous	2 years	Recruiting	NCT01849159
Iran	12	BM-MSC	6×10^7	Single dose	Endobronchial	1 year	Not recruiting	NCT01758055
Mexico	30	AD-MSC	*	Single dose	Intravenous	6 months	Recruiting	NCT01559051

COPD: chronic obstructive pulmonary disease; BMDMC: bone marrow mononuclear derived cells; BM-MSC: bone marrow-derived mesenchymal stem cells; AD-MSC: adipose-derived mesenchymal stem cells. * Data not available.

In Mexico, an open-label, non-randomized, multicenter study is currently ongoing to evaluate the safety and efficacy of autologous adipose-derived stem cell transplantation in GOLD III and IV patients (NCT01559051). Through a liposuction procedure under local anesthesia, adipose tissue specimens are syringe-collected for subsequent processing to isolate the adipose-derived stem cells. At 3-month and 6-month follow-up, the authors intend to assay whether the therapy improved functional capacity and quality of life, to demonstrate that adipose-derived stem cells might also be safe and even effective in COPD, without adverse events at 6 months following therapy.

IDIOPATHIC PULMONARY FIBROSIS

Idiopathic pulmonary fibrosis (IPF) is defined as a chronic, progressive fibrosing interstitial pneumonia of unknown cause. It is seen primarily in older adults, is a condition that is limited to the lungs, and is associated with the histopathological and/or radiologic pattern of usual interstitial pneumonia. Importantly, other known causes of interstitial pneumonia (idiopathic interstitial pneumonias and interstitial lung disease associated with environmental exposure, medication, or systemic disease) must have been ruled out, making IPF a diagnosis of exclusion [Raghu et al., 2011]. The hallmark of IPF—demonstrable both histologically and on high-resolution computed tomography images—is a pattern of areas of fibrosis with scarring and honeycomb changes alternating with areas of less affected or normal parenchyma. These alterations often affect the subpleural and paraseptal parenchyma most intensely, while inflammation is usually mild and is constituted by an irregular interstitial infiltrate of lymphocytes and plasma cells associated with hyperplasia of type 2 alveolar epithelial cells and bronchiolar epithelium.

While there are no definitive studies of the incidence or prevalence of IPF, prevalence estimates have ranged from 2 to 29 cases per 100,000 in the general population [Raghu et al., 2011]. Retrospective longitudinal studies suggest a median survival time of 2–3 years (130–134), and there is no specific treatment. While treatment of conditions such as pulmonary hypertension, gastro-esophageal reflux disease, obesity, emphysema, and obstructive sleep apnea may improve respiratory symptoms in IPF patients, the Committee on Idiopathic Pulmonary Fibrosis does not support the use of any direct pharmacologic therapy for IPF. Conversely, the use of several nonpharmacologic therapies, including oxygen therapy, lung transplantation, mechanical ventilation, and pulmonary rehabilitation, is recommended in appropriate patients.

Preclinical studies using a bleomycin-induced IPF model [Rojas et al., 2005; Cargnoni et al., 2009; Kumamoto et al., 2009; Lee et al., 2010; Bitencourt et al., 2011] demonstrated that stem cells decreased inflammation, with reductions in neutrophil infiltration, fibrosis, and collagen deposition and an increase in epithelial repair [Ortiz et al., 2007; Moodley et al., 2009] (Fig. 2). Due to these positive effects, MSCs are currently in clinical studies in patients with IPF.

Currently, there are three trials officially registered in Clinical-Trials.gov that are taking place to evaluate the safety and feasibility of MSC therapy in IPF patients (Table II). In the United States, a phase I, randomized, blinded and placebo-controlled trial is recruiting 25 IPF patients to investigate the safety, tolerability,

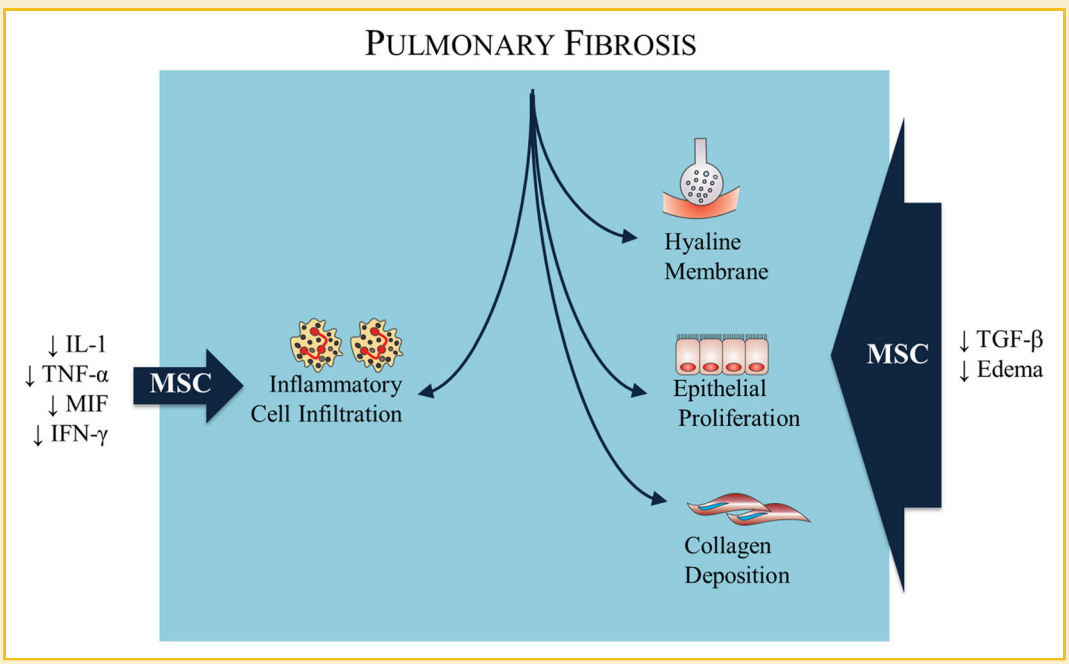

Fig. 2. Potential mechanisms of action of MSCs in pulmonary fibrosis. IL: interleukin; TNF-α: tumor necrosis factor-α; MIF: macrophage migratory inhibitor factor; IFN-γ: interferon gamma; TGF-β: transforming growth factor-β.

TABLE II. Clinical Trials of Mesenchymal Stem Cells in IPF

Disease	Location	Patients	Cell type	Dose	Frequency	Delivery	Follow-up	Status	ClinicalTrials.gov
IPF	USA	25	BM-MSC	2×10^7	Single dose	Intravenous	60 weeks	Recruiting	NCT02013700
	Spain	18	BM-MSC	Escalating doses	*	Endobronchial	12 months	Recruiting	NCT01919827
	Australia	8	Placental MSC	$1–2 \times 10^6$/kg	Single dose	Intravenous	6 months	Not recruiting	NCT01385644

IPF: idiopathic pulmonary fibrosis; BM-MSC: bone marrow-derived mesenchymal stem cells; Placental MSC: placental-derived mesenchymal stem cells. * Data not available.

and potential efficacy of intravenous infusion of allogeneic human MSCs (NCT02013700). After the first month following MSC or placebo infusion, the incidence of any serious adverse events (death, nonfatal pulmonary embolism, stroke, hospitalization for worsening dyspnea, and clinically significant laboratory test abnormalities) will be assessed. This study includes a 60-week follow-up period after MSC (20×10^6 cells) administration to evaluate the impact of MSC transplantation on lung function, quality of life parameters, number of acute IPF exacerbations, and death from any cause.

The migration of MSCs to the injured tissues seems to be central to the efficacy of this cell therapy, raising the possibility that localized inflammatory processes may induce MSCs to release chemokines and growth factors, stimulating tissue repair. Considering that the lung offers the intratracheal/endobronchial route as a direct pathway for drug/cell delivery, this route of administration may potentiate MSC efficacy. Another ongoing trial is being conducted at Navarra University in Spain to examine this issue. This phase I, open-label, multicenter, non-randomized study will evaluate the safety and feasibility of the endobronchial infusion of autologous bone-marrow MSCs at escalating doses in patients with mild-to-moderate IPF (NCT01919827). This study is now recruiting 18 volunteers, which will be followed up for 12 months to determine safety (i.e., incidence of adverse side effects) and efficacy (disease progression as assessed by survival; need for transplantation; deterioration in pulmonary function defined by decline in forced vital capacity $> 10\%$ or in lung diffusion capacity $> 15\%$).

MSCs from sources other than the bone marrow may have therapeutic potential for patients with IPF. Cargnoni and colleagues demonstrated that placenta-derived MSCs might home to the lung and decrease tissue damage induced by bleomycin exposure [Cargnoni et al., 2009]. Based on these experimental studies, in Australia, a phase I, open-label, single-center, non-randomized dose-escalation study intends to evaluate the safety and feasibility of placental-derived MSC infusion in IPF patients (NCT01385644). The study design requires a total of up to eight patients, where the first four will receive one dose of placental MSCs (1×10^6 cells/kg) and be evaluated after 3 months. If no serious adverse events attributable to MSC infusion have occurred in this group, four additional IPF patients will then receive an intravenous infusion of 2×10^6 placental MSCs/kg. Patients will be followed for 6 months after MSC therapy to assess safety and efficacy (assessed via improvement or stabilization of lung function, gas exchange assessment at rest and during exercise, and exercise capacity by the 6MWD test). This trial was expected to complete safety evaluations by May 2013, but so far no outcomes have been published.

SILICOSIS

Silicosis is another important fibrotic pulmonary disease induced by inhalation of particulate silica (SiO_2, silicon dioxide), one of the most abundant minerals on Earth. Silicosis remains a public health problem worldwide, especially in developing countries (e.g., the prevalence of silicosis is 13–31% in South Africa and 22–55% in India) [Abu-Shams et al., 2005]. In its most severe forms, silicosis may progress to respiratory failure and death because of severe gas exchange impairment induced by fibrosis. When silica particles reach the lungs, they accumulate in the respiratory bronchioles and alveoli, and the alveolar macrophages become activated and start to produce high levels of reactive oxygen species and other free radicals [Porter et al., 2006]. Phagocytosis of the silica particles by macrophages leads to local inflammation and nitric oxide release, contributing to inflammation and remodeling, as well as promoting macrophage apoptosis, which triggers a vicious cycle of inflammation. In silicosis, fibroblasts and myofibroblasts are directly involved in the pathogenesis of the fibrosis, with intense collagen deposition. There is no efficacious treatment for silicosis, and in the current scenario, further studies are required to develop new, effective therapies for this disabling disorder. Preclinical studies using an experimental model of silicosis [Lassance et al., 2009; Lopes-Pacheco et al., 2013] demonstrated that BMMCs reduce inflammation, with attenuation in neutrophil infiltration and inflammatory cytokine levels, and reduce fibrosis, with a decrease in collagen deposition. These positive effects encouraged the initial use of BMMCs in a clinical study of patients with silicosis. A non-randomized, phase I trial in patients with chronic and accelerated silicosis, conducted in Brazil, demonstrated that intrabronchial instillation of autologous BMMCs (NCT01239862) is safe. In this study, three patients each received 2×10^7 bone marrow-derived cells labeled with 99mTc. The MSC infusion procedure was well tolerated by the patients, and no respiratory, cardiovascular or hematological complications were observed. Scintigraphy showed an increase in lung perfusion in the basal region up to day 180 after the infusion, while the apex and midzone areas presented reduced perfusion at day 180 [Loivos et al., 2010; Souza et al., 2011]. Besides theses positive outcomes, no clinical study of MSCs in silicosis has been carried out so far.

BRONCHOPULMONARY DYSPLASIA

Bronchopulmonary dysplasia (BPD) is a chronic respiratory disease that results from complications related to the treatment of

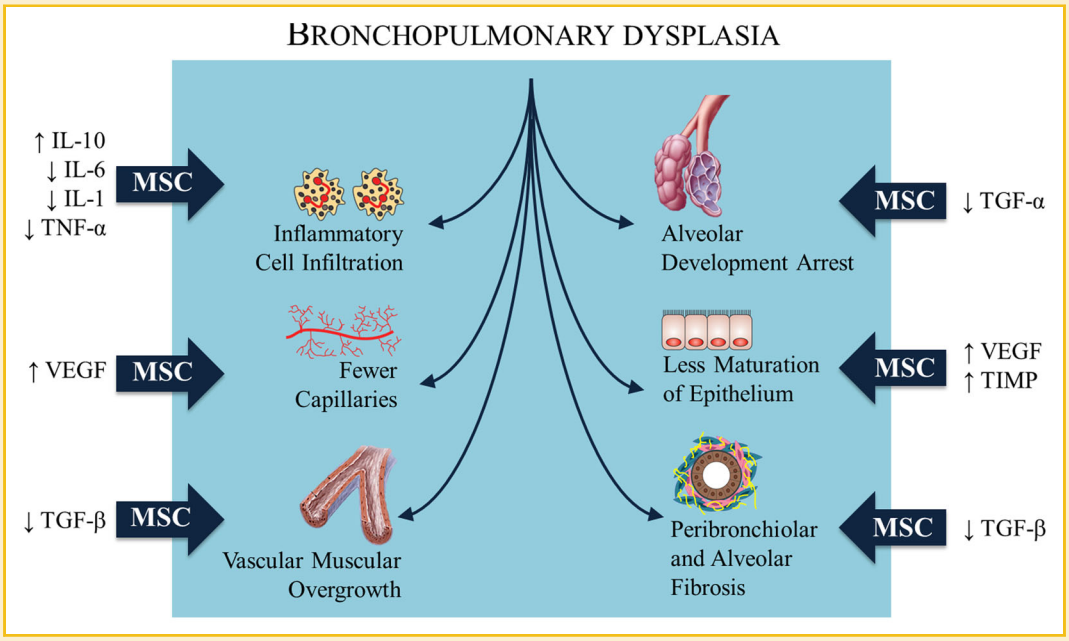

Fig. 3. Potential mechanisms of action of MSCs in bronchopulmonary dysplasia. IL: interleukin; TNF-α: tumor necrosis factor-α; TGF-β: transforming growth factor-β; TGF-α: transforming growth factor-α; VEGF: vascular endothelial growth factor; TIMP: tissue inhibitor of metalloproteinases.

respiratory distress syndrome in low-birth-weight premature infants, or when abnormal lung development occurs in older infants [Walsh et al., 2006]. BPD is clinically diagnosed if there is higher than 21% oxygen dependency for 28 days or more assessed at a postmenstrual age of 36 weeks. It occurs almost exclusively in very preterm infants born before 30 weeks of gestation, when around one in three infants develop BPD [Smith et al., 2005]. The incidence of BPD appears to be rising in parallel with the increased survival rate of very-low-birth-weight infants who are treated for and recover from respiratory distress syndrome. Histologically, BPD is characterized by diffuse pulmonary inflammation, with alveolar and vascular simplification and an arrest of lung development at the late canalicular to early saccular stages. This results in a reduced surface area for gas exchange and chronic pulmonary dysfunction [Thibeault et al., 2003]. The immature lung does not support the increase in respiratory system requirements, requiring supplemental oxygen and mechanical ventilation, triggering inflammation, exacerbating structural deficits, inducing alveolar arrest, and ultimately leading to BPD.

The pulmonary damage induced by BPD is irreversible for many children, and the respiratory impairment initiated during neonatal life may continue into adolescence and adulthood. The most effective therapy for BPD has been its prevention, but pharmacologic approaches have limited efficacy, particularly in more extreme infant prematurity. MSC therapy may have therapeutic potential for infants with BPD, given their immunomodulatory effects over inflammatory mediators as well as their regenerative capacity, either to prevent BPD or to treat infants with established BPD.

Preclinical studies using hyperoxia-induced BPD models have demonstrated that bone-marrow MSC therapy improves alveolar and vascular repair, lung function, and survival in preterm mouse pups [Aslam et al., 2009; van Haaften et al., 2009; Hansmann et al., 2012] (Fig. 3). MSCs may also be isolated from umbilical cord blood (UCB), and evidence from a model of neonatal hyperoxia-induced lung injury in rats suggests that UCB-MSCs may contribute to alveolar repair through paracrine mechanisms similar to those seen with bone-marrow MSC therapy [Chang et al., 2009; Chang et al., 2011]. Since UCB banks are becoming popular worldwide, UCB-MSCs could represent a potential therapeutic tool for BPD, because an individual's own cells could be stored prospectively and used if required. All five ongoing clinical trials of MSC therapy for BPD that have been registered at ClinicalTrials.gov are using PNEUMOSTEM® (MEDIPOST CO., LTD. Seoul) MSCs, which are a human UCB-MSC preparation developed commercially as a potential therapy for premature infants with BPD. All of these trials are or have been carried out in South Korea, and only one has completed to date. This study was an open-label, single-center, phase I clinical study, which evaluated the safety and the efficacy of PNEUMOSTEM® for BPD treatment in premature infants (NCT01297205). Unfortunately, to date, no data from this study have been published.

ACUTE RESPIRATORY DISTRESS SYNDROME

Acute respiratory distress syndrome (ARDS) is a devastating disease process that continues to have a high mortality and for which there is no treatment. The diagnostic criteria for ARDS have recently been revised. In the new definition, the criteria include the following parameters: timing (within 1 week of a known clinical insult or new or worsening respiratory symptoms); chest imaging (bilateral

opacities consistent with pulmonary edema); origin of lung edema: respiratory failure not fully explained by cardiac failure or fluid overload; and oxygenation (different categories according to the degree of hypoxemia severity—mild, moderate, and severe). Approximately 7% of patients admitted to intensive care units will present with or develop ARDS [Force et al., 2012].

ARDS is a major public health problem worldwide, with more than 100,000 cases per year, and carries a high economic burden associated with long intensive care unit and hospital stays. ARDS mortality remains high (30–40%), and no therapy exists for this devastating disease, underlining the urgent need for the development of new approaches. ARDS may result from a variety of disorders and risk factors. Regardless of the cause, the alveolar epithelium and capillary endothelium are affected in ARDS, leading to increased permeability and extravasation of protein-rich fluid into the alveolar space, which compromises surfactant synthesis. The alveolar damage is worsened by alveolar neutrophil influx and by the formation of hyaline membranes, and the injury process can be exacerbated by mechanical ventilation [Force et al., 2012]. In severe cases, the proliferative phase results in an increased number of type II alveolar cells, fibroblasts, myofibroblasts and matrix deposition, fibrosis, and multiple organ failure, which is the leading cause of death in ARDS.

The immunomodulatory and reparative potential of MSCs makes them potential therapeutic tools for the acute inflammatory response to infection and pulmonary injury seen in ARDS. The effects of MSC therapy in relevant preclinical models of ARDS further underline their therapeutic potential. Several preclinical studies of ARDS have demonstrated that MSCs may improve the pulmonary and systemic inflammation characteristic of the disease [Rojas et al., 2005; Gupta et al., 2007; Nemeth et al., 2009; Mei et al., 2010]. In mouse models of LPS-induced ARDS, MSC treatment not only attenuates inflammation by decreasing several inflammatory mediators, including TNF-α, MIP-2, IFN-γ, IL-1β, MIP-1α, IL-6, IL-8, and keratinocyte-derived cytokine in plasma and bronchoalveolar lavage fluid, but also increases secretion of the antibacterial protein lipocalin-2 [Gupta et al., 2012] and is able to rescue epithelial cells with mitochondrial dysfunction by mitochondria transfer [Spees et al., 2006; Islam et al., 2012]. In addition, MSCs favorably influence the host response to bacterial infections, the commonest and most severe cause of ARDS. MSC therapy can reduce bacterial counts via a number of mechanisms, including increased antimicrobial peptide secretion [Krasnodembskaya et al., 2012] and enhanced macrophage phagocytosis [Nemeth et al., 2009]. MSCs also enhance repair following lung injury, as evinced by the findings that both intravenous [Curley et al., 2012] and intratracheal [Curley et al., 2013] MSC therapy restore lung function following ventilator-induced lung injury via a KGF-dependent mechanism. Based on these promising preclinical findings (Fig. 4), a number of early-phase clinical trials have begun to investigate the potential of MSC therapy for severe ARDS.

Currently, two studies of MSC therapy safety in patients with ARDS are ongoing, according to ClinicalTrials.gov. At the University of California, San Francisco, a phase I, multicenter, open-label, dose escalation clinical trial is in progress to assess the safety of intravenous infusion of allogeneic bone marrow-derived human MSCs in ARDS (NCT01775774). The design of this study aims to establish three cohorts with three subjects each who will each receive escalating doses of 1×10^6 cells/kg, 5×10^6 cells/kg, and 1×10^7 cells/kg respectively, to determine if there are any adverse events for each dose, over a follow-up period of 12 months. This ongoing trial, which is recruiting patients with ARDS, is expected to be completed by May 2014. Adipose-derived MSCs have also been used in

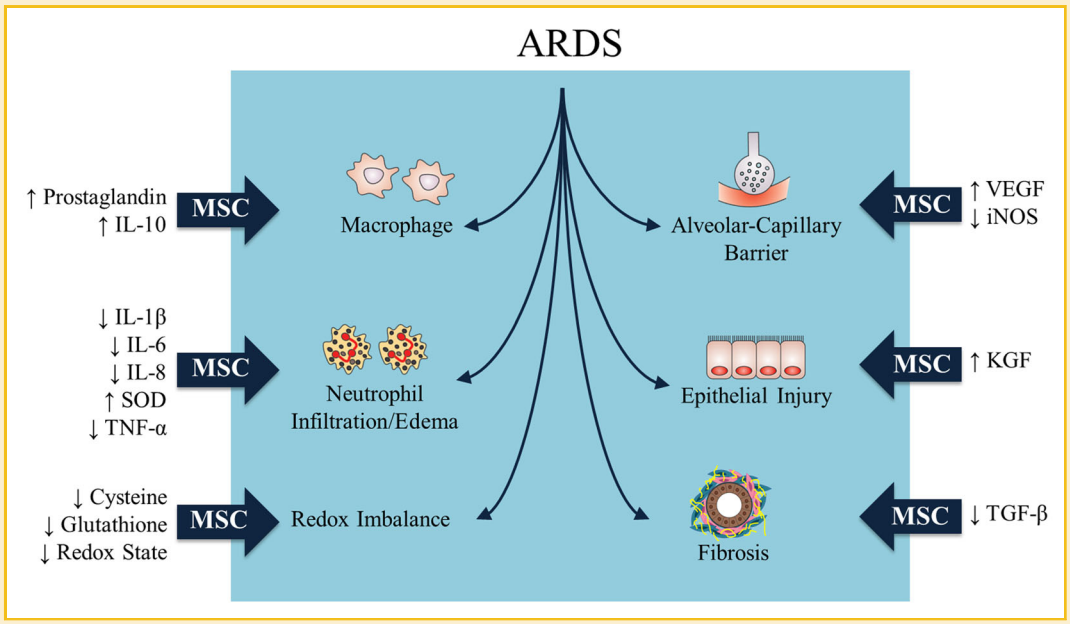

Fig. 4. Potential mechanisms of action of MSCs in acute respiratory distress syndrome (ARDS). IL: interleukin; SOD: superoxide dismutase; TNF-α: tumor necrosis factor-α; VEGF: vascular endothelial growth factor; iNOS: inducible nitric oxide synthase; KGF: keratinocyte growth factor; TGF-β: transforming growth factor-β.

preclinical studies in ARDS. In one such study, Martinez-González and colleagues demonstrated that genetically engineered adipose MSCs overexpressing soluble IL-1 receptor-like-1 decreased lung airspace inflammation and vascular leakage and reduced protein content, differential neutrophil counts, and pro-inflammatory cytokine (TNF-α, IL-6, and macrophage inflammatory protein 2) concentrations in bronchoalveolar lavage fluid [Martinez-Gonzalez et al., 2013]. Subsequently, a phase I, randomized, double-blind, placebo-controlled trial has been carried out in China to test the safety of allogeneic intravenous infusion of human adipose MSCs (1×10^6 cells/kg body weight) in ARDS patients (NCT01902082). This trial is now recruiting patients that will receive the stipulated dose within 48 h of enrollment, and their plasma levels of cytokines and mediators (IL-6, IL-8, TNF-α, surfactant protein-D) will be monitored for 7 days posttreatment.

FUTURE PERSPECTIVES AND CONCERNS

MSCs have generated a great amount of enthusiasm over the past decade as a novel therapeutic strategy for a variety of lung diseases. Although advancements have been made from preclinical studies using MSCs, substantial challenges have yet to be overcome before MSC therapy can be used in clinical practice. Clinical studies published to date have reported that MSC administration is safe, with few adverse effects concerning infusion reactions and late effects. However, due to the relatively small number of patients that have received MSC therapy to date, further investigations should be performed to further characterize its safety profile. Moreover, to ensure MSC quality control, bacteriological tests (to reduce microbial contamination of MSC cultures), viability and phenotype tests, oncogenicity tests, and endotoxin assays should be carefully performed. In addition, the optimal timing and duration of administration, the cell dose (per kilogram of body weight, escalating doses), the source of MSCs, the best delivery route, and the optimal schedule of administration (e.g., single versus repeated doses) all need to be evaluated.

The use of MSCs in the clinical setting requires a large number of cells; however, continuous in vitro passaging of MSCs may result in genetic abnormalities (chromosomal abnormalities, increased c-myc levels and telomerase activity), raising the possibility of cell transformation [Rubio et al., 2005]. MSCs are relatively immune-privileged, as they express low levels of major histocompatibility complex I (MHC-I) molecules and do not express MHC-II molecules or costimulatory molecules such as CD80, CD86, or CD40 [Guo et al., 2009], suggesting that both allogeneic and autologous MSCs can be used in the clinical setting. Nevertheless, further studies with larger sample sizes are required to evaluate which source of cells would result in superior beneficial effects. Recent clinical studies have used MSCs manufactured by different companies and by non-commercial cell repositories; thus, regulations and standards for production of clinical-grade MSCs need to be very well defined and include methods and criteria for MSC culture, storage, shipping, and administration. This is a very important issue, as differences in cell production (e.g., MSC passage) may result in different effects. Answering these emerging questions will enable a more rapid, reliable, and effective translation of MSC therapy from bench to bedside for patients with lung diseases.

Scientists and physicians alike are excited by the therapeutic potential of MSCs for several lung diseases, as suggested by their anti-inflammatory, antifibrotic, antiapoptotic, antibacterial, and pro-reparative features. The early-phase clinical trials conducted to date or currently in progress offer reassurance regarding the safety of MSC therapy for these diseases. Determination of the efficacy of MSC therapies for COPD, silicosis, IPF, BPD, and ARDS will require large-scale, international, multicenter phase III clinical trials. These studies are eagerly awaited.

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