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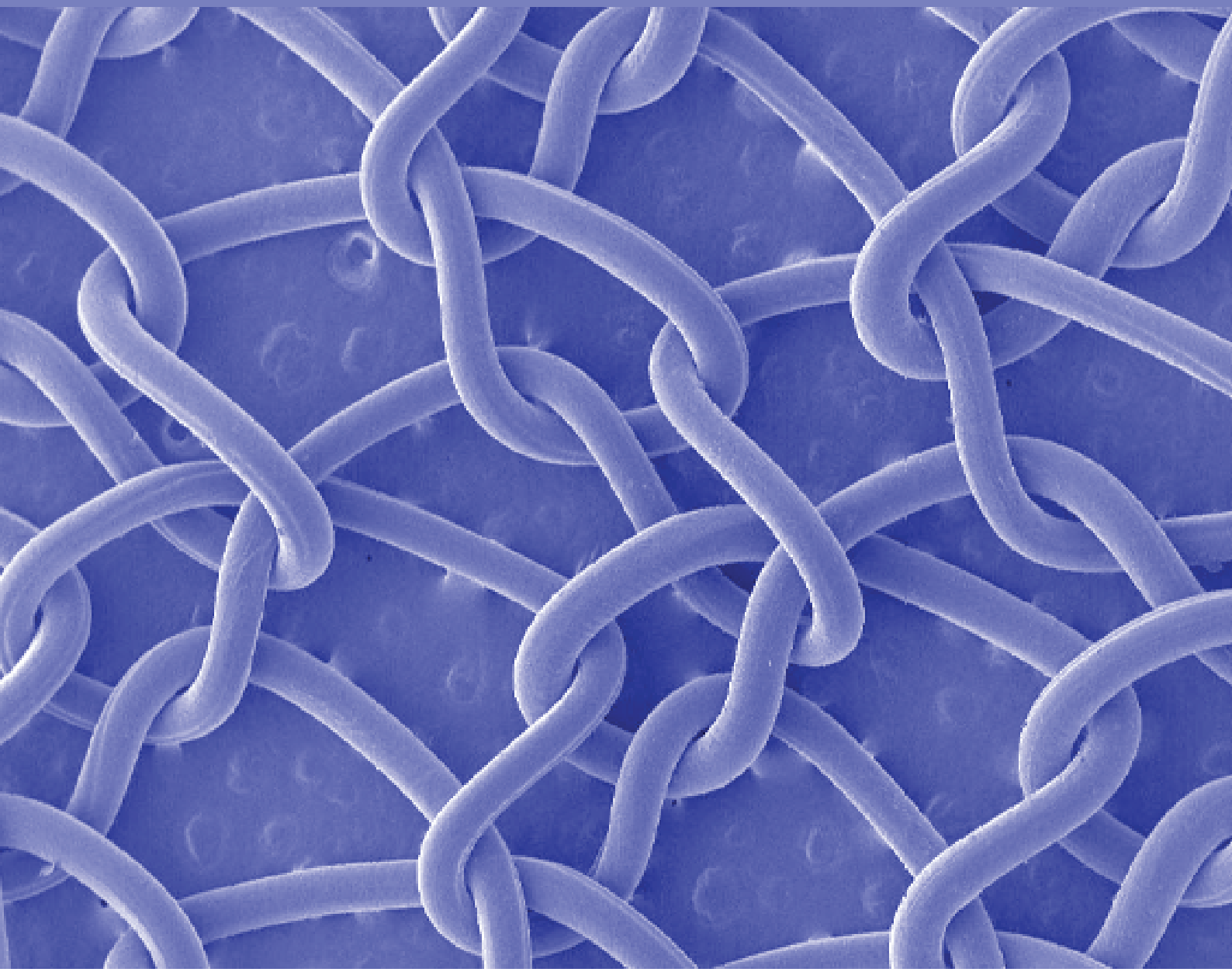

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MESENCHYMAL STROMAL CELLS IN CHRONIC RESPIRATORY DISEASES: WHAT'S NEW?

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ABSTRACT

Chronic respiratory diseases, including asthma and chronic obstructive pulmonary disease (COPD), are a major health issue worldwide due to their growing prevalence and high economic costs, which include prolonged medication use and frequent hospitalizations. Both asthma and COPD are incurable, and each is characterized by chronic inflammation, tissue remodeling, and ultrastructural alterations that might not be amenable to available therapies. Mesenchymal stromal cells (MSCs) are non-hematopoietic, immunosuppressive cells found in bone marrow, adipose tissue, placenta, and other tissues and have demonstrated anti-inflammatory actions in a number of preclinical models of asthma and COPD. Although the entire repertoire of action of MSCs has not been elucidated, there is growing interest in the potential clinical utility of MSC-based cell therapy in chronic respiratory diseases. The present review will focus on the most recent mechanisms that have been elucidated from preclinical studies in asthma and COPD. In addition, a comprehensive review of clinical trials conducted to date will be presented.

Keywords: mesenchymal stromal cells; COPD; asthma; acute respiratory distress syndrome; pulmonary arterial hypertension.

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Introduction

Mesenchymal stromal cells (MSCs) are undifferentiated, clonogenic cells that may be found in several adult organs, especially in bone marrow. MSCs have been classically isolated and characterized by a set of criteria: 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, or HLA-DR; and ability to differentiate into adipocytes, chondrocytes, and osteocytes *in vitro* [1]. Because of their low expression of major histocompatibility complex molecules and absence of co-stimulatory molecules, as well as their ability to release several soluble mediators, MSCs are strong candidates for use in cell-based therapy approaches for a number of immune and inflammatory diseases. They are currently believed to operate by modulating injured cell function through two main mechanisms, namely (1) cell-dependent contact and (2) paracrine mechanisms, which involve the production of soluble mediators or the transfer of cellular materials such as proteins, nucleic acids, and cell organelles via microvesicles [2, 3].
There is growing interest in the potential use of MSCs in respiratory diseases. This mini-review will focus on current knowledge involving the application of MSCs as therapy for asthma and chronic obstructive pulmonary disease (COPD), the two leading chronic respiratory diseases, which lead to numerous hospitalizations every year and carry high mortality rates.

Asthma

Asthma is a chronic disease characterized by reversible airway obstruction, airway inflammation, and tissue remodeling. Airway inflammation in asthma is a multicellular process involving mainly eosinophils, neutrophils, CD4 T lymphocytes, and mast cells. Inhaled glucocorticoids have been widely used to suppress airway inflammation in asthmatics, but may cause several side effects when used in high doses or for prolonged periods. In response to chronic injury/inflammation, asthma patients develop airway remodeling, which is marked by progressive structural modifications in the composition, content, and organization of the cellular and molecular constituents of the airway wall and lung parenchyma. Although current asthma therapies (e.g., corticosteroids, anti-leukotrienes, and theophylline) are effective in reducing inflammation, pulmonary remodeling is poorly if at all responsive. In mild allergic asthma, inflammation is generally an antigen-stimulated, Th2-mediated, eosinophil-driven process. In more severe asthma, particularly in corticosteroid-resistant asthma, Th17-mediated, neutrophilic-driven inflammation plays a more prominent role. Interfering with these immune-driven processes is a promising area for development of new therapeutic approaches.
A growing number of preclinical studies, mostly in mouse models of allergic airways inflammation, are demonstrating the efficacy of MSC administration during either antigen sensitization or challenge in ameliorating lung inflammation and airway hyperresponsiveness (Table 1). A number of potential mechanisms of action of MSCs have emerged, including reduction of Th2 cytokines (interleukin [IL]-4, IL-5, IL-13) [4-8], and increases in counter-regulatory Th1 (interferon gamma, IFN-γ) [4, 9, 10] and regulatory and anti-inflammatory mediators, including IL-10, IL-12, IL-1 receptor antagonist, indoleamine 2,3-dioxygenase (IDO), transforming growth factor (TGF-β), and prostaglandin E2 (PGE2) [4, 5, 10-13]. Specific effects of MSC administration include attenuation of hallmark features including airway hyperresponsiveness,

inflammatory cell infiltration, mucus production in the lung, goblet cell hyperplasia, and pulmonary tissue remodeling (collagen deposition, airway muscularization). In animal models, MSCs can further modulate the activity of other innate and adaptive immune cells, such as dendritic cells and T cells [5, 6, 14].

Table 1. Experimental studies with mesenchymal stromal cells in asthma

First Author/Year	Protocol	Model	Cell Type	Route	Frequency	Dose
Duong, 2015	HDM	BALB/c	BM-MSC	i.v.	Single dose	$1x10^6$
Lathrop, 2014	AHE	C57BL/6	BM-MSC	i.v.	Two doses	$1x10^6$
Mariñas-Pardo, 2014	HDM	BALB/c	AD-MSC	i.v.	Single dose	$3x10^5$
Song, 2015	OVA	NOD/SCID	Human BM-MSC	i.v.	Single dose	$1x10^6$
Lee, 2011	TDI	BALB/c	Rat BM-MSC	i.v.	Single dose	$1x10^5$
Ge, 2013	OVA	BALB/c	BM-MSC	i.t.	Single dose	$5x10^5$
Sun, 2012	OVA	BALB/c	iPSC-MSC, BM-MSC	i.v.	Single dose	$1x10^6$
Bonfield, 2010	OVA	BALB/c	Human BM-MSC	i.v.	Single dose	$1x10^6$
Ge, 2013	OVA	BALB/c	BM-MSC	i.t.	Single dose	$5x10^5$
Abreu, 2013	OVA	C57BL/6	BM-MSC	i.v.	Single dose	$1x10^5$
Nemeth, 2010	RW	C57BL/6	BM-MSC	i.v.	Single dose	$7.5x10^5$
Goodwin, 2011	OVA	C57BL/6	BM-MSC	i.v.	Two doses	$2x10^6$
Mathias, 2013	OVA	BALB/c	Human BM-MSC, AD-MSC and UC-MSC	i.v.	Three doses	$1x10^6$
Cho, 2014	OVA	C57BL/6	AD-MSC	i.v.	Four doses	$1x10^6$

AHE: *Aspergillus fumigatus* hyphal extract; HDM: house dust mite; OVA: ovalbumin; RW: ragweed challenge; TDI: toluene diisocyanate; BM: bone marrow; AD: adipose tissue; UC: umbilical cord; iPSC: induced pluripotent stromal cells; MSC: mesenchymal stromal cells.

One of the key postulated mechanisms investigated is suppression of Th2-driven allergic responses and recruitment of regulatory T cells (Tregs) to the lungs. Tregs are a unique T-cell population with strong immunosuppressive properties. $CD4^+CD25^+$ Tregs play a protective role in suppressing airway eosinophilic inflammation and the development of airway hyperreactivity in asthma. Ge et al. demonstrated that the frequency of $CD4^+CD25^+$ Tregs in pulmonary lymph nodes was increased markedly by administrating bone marrow-derived MSCs (BMSCs) in a murine model of ovalbumin (OVA)-induced asthma, and suggested that MSCs exert anti-inflammatory effects by upregulation of Tregs with accompanying increased production of IL-10 [11]. Moreover, IFN-γ production has been shown to inhibit Th2-mediated allergic airway inflammation in several models. In asthma, modulation of the Th1 cytokine IFN-γ by MSC administration remains controversial [4, 9, 12, 15]. Reports have shown distinct behaviors of MSCs obtained from different sources. BMSC administration appears to increase IFN-γ levels in conditioned media from splenic CD4 T lymphocytes [9] and systemic levels thereof [15] in a chronic asthma model, while attenuating IFN-γ levels in bronchoalveolar lavage fluid (BALF) in the same model [15]. Conversely, adipose-derived MSCs (ADSCs) have been shown to induce increases in IFN-γ levels in BALF and lung-draining lymph nodes (LLN) and in the number of IFN-γ^+ $CD4^+$ T cells in the LLN of asthmatic mice, which occurred concomitantly with an increased ratio of Tregs [4]. In a time course analysis, Mariñas-Pardo et al. demonstrated that increased IFN-γ levels are observed in BALF 72h after ADSC injection in a murine model of asthma induced by house dust mites (HDM), but, at 2 weeks, IFN-γ had returned to levels similar to those of a control group [10]. At this point, despite good evidence that MSCs act by increasing Treg cells and decreasing Th2-mediated inflammation, the role of IFNγ remains unclear.

MSC therapy has the potential to play an essential role in treatment of the 5-10% of asthmatics with severe disease that is poorly controlled clinically and resistant to corticosteroids. These patients account for the high rates of morbidity, mortality, and health care expenditures induced by asthma. In many of these patients, asthma is driven by interleukin-17 (IL-17)-mediated neutrophilic airway inflammation, which, in turn, is driven by antigen-specific Th17 CD4 T cells. In a murine model of Th17-mediated neutrophilic severe allergic airway inflammation induced by *Aspergillus fumigatus* hyphal extract (AHE), the mechanism of action involved in the beneficial effects of MSCs seemed to be distinct from those observed in preclinical models of Th2-mediated eosinophilic allergic airway inflammation [16]. Lathrop et al. demonstrated in both acute and recurrent AHE exposures that systemic MSC administration during antigen challenge reduced Th2- and Th17-induced inflammatory mediators, whereas a decrease in Th17-mediated IL-17a production by antigen-specific Th17 T cells appeared to be a key mechanism. No significant change in the number of splenic $CD4^+CD25^+$ regulatory T cells was observed in either AHE model [16]. Cruz et al. demonstrated that the BMSC effects in this model occurred both with freshly thawed frozen MSCs and with continuously cultured MSCs, an important observation for potential clinical use [16]. This study also demonstrated that human BMSCS were as effective as mouse BMSCs in an immunocompetent mouse model, providing a more powerful preclinical model for the study of human MSC actions. Recent evidence also shows that human and mouse BMSC-derived conditioned medium or extravesicle particles alone could reduce AHE-induced airway hyperresponsiveness, lung inflammation, and antigen-specific CD4 T cell Th2/Th17 phenotype C [17]. Although the mechanisms of action are not yet known, this opens up another potential therapeutic approach in which MSCs themselves may not be

necessary.

Other recent studies have identified macrophages as key cells in the beneficial effects of MSCs in preclinical asthma models. Mathias et al. found that *in vivo* depletion of alveolar macrophages abrogated the effects of human ADSCs on allergic inflammation and airway hyperresponsiveness in a murine model of allergic inflammation induced by OVA [12]. Specifically, when mice were previously treated with clodronate-encapsulated liposomes to deplete alveolar macrophages, ADSCs were unable to restore airway resistance and compliance or to reduce mucus-secreting goblet cells and inflammatory cell infiltration [12]. Macrophages are a heterogeneous cell population, capable of responding to local signals to change their effector function. Classically activated M1 macrophages are activated by IFN-γ and toll-like receptor ligands to mediate host defense, while alternatively activated M2 macrophages are activated by IL-4 or IL-13 to promote anti-inflammatory functions and tissue repair [18]. Co-culture studies have demonstrated that BMSCs lead macrophages to a M2 phenotype polarization associated with high IL-10 synthesis [19]. Lung tissue isolated from ADSC-treated mice following intranasal OVA challenge demonstrated increased IL-10 levels, which was significantly inhibited by prior depletion of alveolar macrophages. However, there was no evidence of M1/M2 switching induced by ADSC treatment [12]. Nevertheless, in other studies, BMSC administration led to TGF-β driven M2 polarization in alveolar macrophages, which seemed to play a critical role in inhibiting hallmark features of asthma [13].

Other cell types may also be potentially useful in asthma. A recent publication demonstrated that syngeneic bone marrow-derived mononuclear cells (BMDMCs) might be more effective than MSCs in a mouse model of allergic asthma with airway remodeling [20]. Notably, systemic administration of BMDMCs during antigen challenge resulted in a greater recovery of lung architecture and function, less parenchymal collagen deposition, and greater reduction of growth factors related to pulmonary fibrosis and angiogenesis than did MSC administration [20]. This raises the possibility of using autologous BMDMCs in asthmatic patients. However, so far, there have been no clinical investigations of either MSCs or BMDMCs in patients with asthma.

Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is a major global public health challenge, predominantly caused by cigarette smoking, and is projected to become the third leading cause of death worldwide by 2020. In COPD, the major mechanisms underlying chronic airflow limitation are the irreversible loss of alveolar architecture, airspace enlargement, destruction of the lung parenchyma, and loss of elastic recoil. Unfortunately, COPD remains incurable except by lung transplantation. However, a number of preclinical trials in mouse models of cigarette smoke exposure, elastase instillation, and other models of COPD have demonstrated protective and sometime reparative effects of MSC administration (Table 2). This raises the possibility that MSCs may have clinical utility in COPD.

Table 2. Experimental studies with mesenchymal stromal cells in emphysema

First Author/Year	Protocol	Model	Cell Type	Route	Frequency	Dose
Kim, 2014	PPE	C57BL/6	Human AD-MSC	i.v.	Single dose	$5x10^5$
Li, 2014	CSE+LPS	SD rats	AF-MSC	i.t.	Single dose	$4x10^6$
Li, 2014	CSE	SD rats	Human iPSC-MSC and BM-MSC	i.v.	Two doses	$3x10^6$
Guan, 2013	CSE	SD rats	BM-MSC	i.t.	Single dose	$6x10^6$
Gu, 2015	CSE	SD rats	BM-MSC	i.t.	Twice per week	$6x10^6$
Zhen, 2010	Papain	Lewis rats	BM-MSC	i.v.	Single dose	$4x10^6$
Zhao, 2014	LPS	Wistar rats	BM-MSC	i.v.	Single dose	$5x10^6$
Huh, 2011	CSE	Lewis rats	BM-MSC	i.v.	Single dose	$6x10^5$
Ingenito, 2012	PPE	Sheep	L-MSC	i.t.	Five doses	$5x10^7$/ site
Antunes, 2014	PPE	C57BL/6	BM-MSC, AD-MSC and L-MSC	i.v./i.t.	Single dose	$1x10^5$

PPE: pancreatic porcine elastase; CS: cigarette smoke extract; LPS: lipopolysaccharide; SP: Sprague-Dawley rats; BM: bone marrow; AD: adipose tissue; AF: amniotic fluid; iPSC: induced pluripotent stromal cells; L: lung; MSC: mesenchymal stromal cells.

The mechanisms of MSC actions in these COPD models also seem to involve the paracrine release of anti-inflammatory, anti-apoptotic, and pro-angiogenic mediators in the absence of any significant engraftment of MSCs in the lung. Tracking ADSCs using fluorescence optical imaging with quantum dots in a preclinical model of elastase-induced emphysema demonstrates that these cells may only be detected at significant levels in the lungs up to 24h following systemic administration [21]. However, the beneficial effects of MSC administration, such as reductions in cell apoptosis and mean linear intercept and increased angiogenesis, may be sustained for longer periods (e.g., 2 months) in both cigarette smoke and elastase-induced emphysema models [22-24]. More recent studies have demonstrated that systemic administration of MSC-conditioned medium leads to similar repair of emphysematous areas, with increases in the number of small pulmonary vessels. Although the specific content of the conditioned medium including the potential role of microvesicle particles, remains to be elucidated, these data demonstrate that the secretome produced by MSCs is sufficient to ameliorate elastase/smoke-induced emphysema [23, 25]. Other studies have shown that systemic MSC administration can

downregulate levels of several pro-inflammatory mediators and proteases in lung, in parallel with upregulation of anti-inflammatory mediators and growth factors related to epithelial and endothelial cells recovery, as well as reduce pulmonary epithelial/endothelial cell apoptosis in emphysema models [22, 23, 25-29].

VEGF upregulation appears to be a major mechanism of MSC effects in preclinical models of emphysema [22, 27, 29]. VEGF is a pluripotent growth factor that is critical for endothelial cell proliferation and lung development and is implicated in several lung disorders, including COPD: reduced VEGF might result in pulmonary endothelial cell death and subsequently impair normal microcirculation and epithelial cell repair. Different sources of MSCs are able to increase VEGF production after transplantation in emphysema; however, BMSCs have been shown to be the most efficient source [22]. VEGF upregulation has been related to decreased pulmonary epithelial/endothelial apoptosis [22, 27, 29] and to the alleviation of extrapulmonary effects in elastase-induced emphysema, including pulmonary arterial hypertension and increased right ventricle area [22].

Macrophages are key effector cells in the pathophysiology of emphysema. Gu et al. (2015) recently demonstrated that MSCs attenuate airway inflammation in mice by suppressing cigarette smoke (CS)-induced cyclooxygenase-2 (COX-2) expression and COX-2-mediated prostaglandin E2 (PGE2) production in macrophages, via inhibition of p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) pathways [26]. Double immunofluorescence staining revealed an increase in $COX\text{-}2^{+}CD68^{+}$ macrophages in the lungs of CS-exposed mice, while a significant reduction in COX-2 protein levels and a slight reduction in $CD68^{+}$ macrophages occurred after MSC administration [26]. Accordingly, recent data support MSC-induced M2 macrophage polarization in different experimental models of emphysema; a significant increase in the $IL\text{-}10^{+}CD68^{+}$ macrophage population in the BALF of CS-exposed mice was reported [26], while an increased number of $arginase\text{-}1^{+}$ macrophages and reduced $iNOS^{+}$ macrophages was observed in the lungs in a murine model of elastase-induced emphysema [22].

CS-induced mitochondrial dysfunction in murine lungs has been shown to play a critical role in CS-induced emphysema. Mitochondrial transfer from BM-MSCs to epithelial cells, endothelial cells, and cardiomyocytes has been observed *in vitro* and *in vivo* [2]. More recently, Li et al. demonstrated mitochondrial transfer *in vivo* from human induced pluripotent stromal cell-derived MSCs (iPSC-MSCs) and BM-MSCs to the host cells of damaged mouse lung tissue after CS exposure [30]. Mitochondrial transfer is enhanced by CS exposure and depends on the MSC source. Notably, iPSC-MSCs have a higher transfer rate, mediated by formation of tunneling nanotube-like structures, and greater ability to rescue the ATP levels of bronchial epithelial cells *in vitro* compared to BMSCs [30]. The mechanisms underlying the differential ability of MSCs obtained from different sources to promote cell-cell connections and transfer mitochondria remain unclear at present.

Clinical Trials of MSCs in COPD: an Update

Several clinical trials have been initiated in COPD/emphysema patients based on the promising results obtained from preclinical studies with MSCs. However, few of these have published findings to date. The first safety trial in COPD registered in ClinicalTrials.gov (NCT01110252), conducted in Brazil, used BMDMCs (which contain MSCs). The 3-year follow-up of the four patients/volunteers with advanced COPD (stage IV dyspnea) infused with BMDMCs revealed no obvious short- or long-

term adverse effects attributable to treatment [31, 32]. However, this was an uncontrolled, open-label trial, and no conclusions about potential efficacy can be drawn.

In the U.S., the results of a clinical trial (NCT00683722) using intravenous allogeneic MSCs (Prochymal®; Osiris Therapeutics Inc.) has been published [33]. Sixty-two patients were randomized to receive 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. Endpoints included a comprehensive safety evaluation, pulmonary function testing (PFT), and quality-of-life indicators including questionnaires, 6-min walk test (6MWT), and assessments of systemic inflammation. The study found that MSC therapy appears to be safe in COPD, with no infusion-related toxicity and no attributable deaths or serious adverse events. Moreover, a subgroup of patients who had elevated circulating C-reactive protein levels at baseline exhibited a significant decrease following MSC infusion [33].

In Brazil, a phase I, non-randomized, open-label study was recently completed (NCT01872624). Ten patients with severe heterogeneous emphysema were allocated into two main groups to receive bronchoscopic administration of allogeneic BMSCs or saline before insertion of one-way endobronchial valves. A 4-month follow-up to test the safety of the procedures, as assessed by evaluations of quality of life, pulmonary function, and inflammatory status (C-reactive protein and erythrocyte sedimentation rate, complete blood count in peripheral blood) is in progress; however, no data have been published yet.

In the Netherlands, a phase I, non-randomized, non-blinded, prospective study to test the safety and feasibility of BMSC administration prior to and after lung volume reduction surgery (LVRS) for severe pulmonary COPD has been concluded (NCT01306513). In this trial, 10 patients with COPD (GOLD III) received two intravenous infusions of autologous BMSCs after one-sided LVRS and prior to a second LVRS (3 and 4 weeks before) in the contralateral lung. The investigators expect to observe differences in days between post-surgical transpleural air leak of the lung in each patient after the first LVRS (before BMSC administration) and second LVRS (3 weeks after the last intravenous BMSC dose), as well as different histological responses in resected lung tissue (measured by immunohistochemistry of markers of inflammation, fibrosis, and repair). This study has apparently been completed, but thus 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 systemic administration of allogeneic BMSCs (NCT01849159). In this study, 2×10^8 MSCs (hypoxia pre-conditioned in 1% oxygen) will be administered to patients with severe COPD every 2 months over a 1-year period. This study, which is currently recruiting patients, will employ three different efficacy endpoints (lung tissue density measured by CT densitometry, pulmonary function, and diffusing capacity [DLCO]), assessed 6, 12, and 24 months following randomization. The estimated completion date is June 2016.

In Miami, Florida, an open-label, non-randomized, multicenter study is currently recruiting about 100 patients to evaluate the safety and efficacy of systemic administration of autologous adipose-derived stromal cells in GOLD III and IV patients (NCT02041000). At 6-month follow-up, the authors intend to assess functional capacity and quality of life to demonstrate that adipose-derived stromal cells might also be safe and even effective in COPD, without short-term adverse events. This study has an estimated completion date of January 2016.

Conclusion

The potential therapeutic use of mesenchymal stromal cells for asthma and emphysema treatment is still a source of controversy. The available preclinical data support use of MSCs for chronic pulmonary diseases such as asthma and COPD, and suggest a number of potential mechanisms of action, including anti-inflammatory, anti-fibrotic, anti-apoptotic, and pro-angiogenic effects (**Figure 1**). However, preclinical models may not truly reflect human pathogenesis, and only further clinical investigation can determine the potential efficacy of MSC administration in asthma and COPD. Importantly, the early-phase trials that have been conducted to date have consistently demonstrated that MSC administration appears safe in patients with these diseases. Additional preclinical mechanistic studies and larger-scale Phase II and III efficacy trials are still necessary before MSCs becomes a viable therapeutic approach for either asthma or COPD.

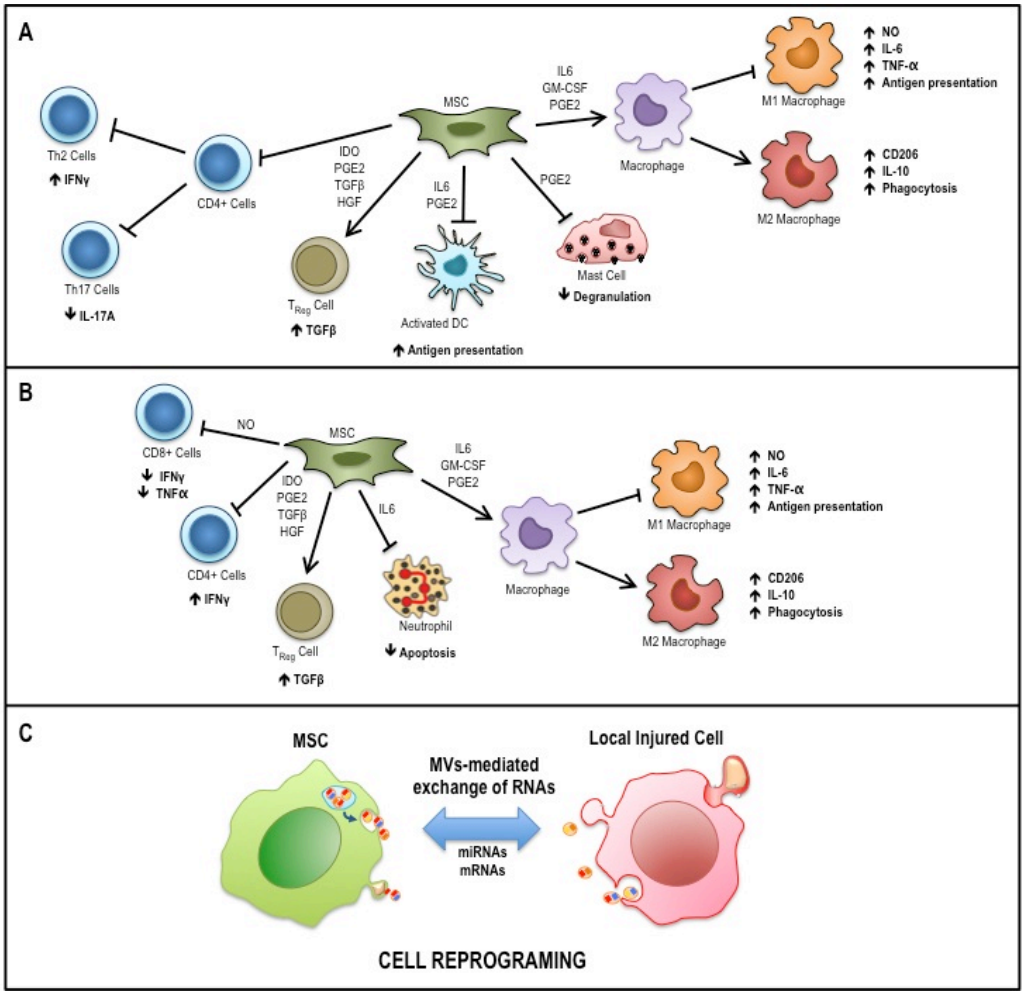

Figure 1. Mechanisms of action of mesenchymal stromal cells (MSC). (A) Immunosuppression of immune cells in asthma: MSC-derived prostaglandin E2 (PGE2), IL-6, and granulocyte macrophage colony-stimulating factor (GM-CSF) affect macrophage function, contribute to the suppression of macrophage M1 polarization, and stimulate M2 polarization; MSCs inhibit dendritic cell (DC) activation, differentiation, and effector functions also mediated by IL-6 and GM-CSF; MSCs produce soluble factors (indoleamine 2,3-dioxygenase – IDO, transforming growth factor-β – TGF-β, hepatocyte growth factor – HGF, PGE2) that suppress $CD4^+$ cells and activate the Treg cell population; MSC-derived PGE2 also contributes to inhibition of mast cell degranulation. (B) Immunosuppression of immune cells in COPD: MSC-derived prostaglandin E2 (PGE2), IL-6, and granulocyte macrophage colony-stimulating factor (GM-CSF) affect macrophage function, contribute to the suppression of macrophage M1 polarization, and stimulate M2 polarization; MSC-derived nitric oxide (NO)

production induces suppression of CD8+ cells, while IDO, TGF-β, HGF, and PGE2 production suppress $CD4^+$ cells and activate the Treg cell population; MSC-derived IL-6 production inhibits neutrophil function. (C) Extracellular vesicle-mediated RNA exchange: transfer of genetic information from MSCs (miRNAs, mRNAs) may activate regenerative programs in local injured cells, with activation of tissue self-repair mechanisms; rare RNA transfer from recipient cells might induce MSC differentiation. IL-10: interleukin 10; IL-17A: interleukin 17A; TNFα: tumor necrosis factor alpha; IFNγ: interferon gamma.

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