Mesenchymal stromal cells: a novel therapy for the treatment of chronic obstructive pulmonary disease?

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ABSTRACT

COPD is characterised by tissue destruction and inflammation. Given the lack of curative treatments and the progressive nature of the disease, new treatments for COPD are highly relevant. In vitro cell culture and animal studies have demonstrated that mesenchymal stromal cells (MSCs) have the capacity to modify immune responses and to enhance tissue repair. These properties of MSCs provided a rationale to investigate their potential for treatment of a variety of diseases, including COPD. Preclinical models support the hypothesis that MSCs may have clinical efficacy in COPD. However, although clinical trials have demonstrated the safety of MSC treatment, thus far they have not provided evidence for MSC efficacy in the treatment of COPD. In this review, we discuss the rationale for MSC-based cell therapy in COPD, the main findings from in vitro and in vivo preclinical COPD model studies, clinical trials in patients with COPD and directions for further research.

INTRODUCTION

Mesenchymal stromal cells (MSCs) are cells of non-haematopoietic origin, with the capacity to differentiate into multiple lineages of the mesenchyme, that is, chondrocytes, osteoblasts and adipocytes. Although an absolute definition of MSCs is not available, the currently used working criteria from the International Society for Cellular Therapy suggest defining MSCs on isolation by (i) their adherence to plastic; (ii) expression of CD73, CD90 and CD105 on their cell surface; (iii) absence of several haematopoietic and endothelial markers (ie, CD45, CD34, CD11b or CD14, CD79 or CD19 and HLA-DR in human MSCs).[1] Unique MSC-specific markers have not yet been identified, and MSCs constitute a heterogeneous cell population, including both multipotent (stem) cells and progenitor cells and might even contain pluripotent cell fractions.[2] MSCs were first described in the bone marrow where they constitute a small fraction of cells (0.001%–0.01%) that closely interact with haematopoietic cells to support haematopoiesis and skeletal homeostasis.[3 4] Since then, it has become evident that MSCs reside in many tissues, including mesenchymal tissues (bone, adipose tissue, connective tissue), umbilical cord and several organs including the liver, spleen and lung.[5] Functional in vitro assays indicate different physiological roles of MSCs related to their heterogeneity and tissue location of origin.[6–8]

On infusion, culture-expanded MSCs regulate inflammatory and immune responses and tissue repair. Following early observations that MSCs inhibit T-cell proliferation,[9] MSCs were found to interact with the majority of innate and adaptive immune cells.[10] MSCs can respond to local triggers, such as inflammatory cytokines and pathogen-associated and damage-associated molecular patterns. These triggers functionally mature MSCs towards either a pro-inflammatory or anti-inflammatory phenotype to regulate inflammation.[11 12] MSCs furthermore contribute to tissue homeostasis through anti-apoptotic and regenerative properties.[13] These various effects can be mediated via cell-to-cell interactions and secretion of soluble factors including growth factors, matrix proteins and cytokines, and through mitochondrial transfer and secretion of extracellular vesicles.[14 15] Finally, transdifferentiation and engraftment of MSCs into local tissue have been described,[16 17] but it is unclear to which extent this contributes to putative repair-enhancing activities of infused MSCs.

These largely preclinical observations suggest that MSCs exert a wide range of activities that may be beneficial clinically, but how they relate to MSC activity in humans is incompletely understood. The first clinical trials in the late 90s[18] assessed safety of MSCs in non-haematopoietic diseases. The clinical potential of MSCs was put in the spotlight by a landmark case report by Le Blanc *et al* in 2004, indicating MSC efficacy on immune restoration in a paediatric patient with refractory graft-versus-host disease.[19] This boosted the interest in MSC-based cell therapy for a variety of diseases characterised by dysregulated immune responses (inflammation) and/or by tissue damage (eg, ischaemic heart disease, spinal cord injury, osteogenesis imperfecta). In 2016, a phase III clinical trial reported positive results for the treatment of therapy-resistant complex perianal fistulas in Crohn's disease.[20] Thus far, clinical trials have indicated that MSC administration is safe and have shown promising results in immune-related disorders but mixed results regarding the clinical benefit in other diseases.[21 22] The field is cautiously advancing towards placebo-controlled trials to further evaluate the efficacy of MSCs and research is ongoing to improve treatment efficacy and study the therapeutic potential of MSCs in other patient groups.

Preclinical data indicate effectiveness of MSCs for treatment of a variety of respiratory diseases, including pulmonary hypertension, asthma, bronchiolitis obliterans, idiopathic pulmonary fibrosis (IPF), acute respiratory distress syndrome (ARDS) and bronchopulmonary dysplasia (BPD).[23–25] Clinical trials in so far limited numbers of patients with IPF, ARDS or BPD have revealed that administration of MSCs (intravenous or intratracheal) is safe but have not yet demonstrated clinical benefit from MSC administration.[26–28] Because COPD is characterised by inflammation, airway remodelling and destruction of lung architecture,[29] the clinical potential of a cell population that can induce an anti-inflammatory, regenerative environment seems obvious. Indeed, supported by preclinical studies and based on promising results in immune diseases, MSCs have already been investigated in patients with COPD. Here, the data from these (pre)clinical studies using MSC-based cell therapy will be summarised, subdivided by data from in vitro, in vivo and clinical studies. Cell therapy studies using bone marrow cells that were not further cultured and/or selected before administration are not discussed in this review.

EFFECTS OF MSCs IN LUNG INJURY MODELS IN VITRO

This section will provide a non-exhaustive overview of in vitro studies focussing on effects of MSCs on inflammation and repair using lung epithelial or endothelial cell injury models. For a broader perspective on the anti-inflammatory, regenerative and paracrine effects of MSCs, we refer to the reviews by (Uccelli and de Rosbo,[10] Murphy *et al*.[11] and Liang *et al*.[14]).

Anti-inflammatory effects

Anti-inflammatory effects of MSCs have been extensively studied in vitro.[10] These include effects of MSCs on cells of the innate and adaptive immune system, and modulation of the balance between proteases and protease inhibitors. Immunomodulating effects of MSCs include inhibition of T-cell and B-cell proliferation, induction of regulatory B-cells and T-cells and skewing of monocyte/macrophage and dendritic cells to anti-inflammatory and tolerogenic phenotypes. These effects are exerted through direct cell-to-cell contact (eg, via programmed death-1 for MSC and T-cell interactions), changes in amino acid and lipid metabolism through indolamine 2,3-dioxygenase expression and COX2-mediated prostaglandin E2 (PGE2) production and secreted factors including transforming growth factor-β (TGF-β) and hepatocyte growth factor (HGF). Recent data suggest that phagocytosis of apoptotic MSCs by macrophages, and subsequent polarisation to an alternative macrophage phenotype contributes to their immunoregulatory effects.[30 31] MSCs were also found to induce expression of secretory leucocyte protease inhibitor in elastase-treated lung epithelial cells via MSC-secreted epidermal growth factor (EGF) and HGF.[32] This response is likely beneficial, especially in COPD, as protease inhibitors counteract protease-mediated tissue injury and degradation of protective mediators.[33] In cocultures with cigarette smoke extract (CSE)-stimulated macrophages, MSCs increased the viability of macrophages and decreased their expression of the pro-inflammatory mediators cyclooxygenase 2 (*COX2*), interleukin (*IL*)-6 and inducible nitric oxide synthase, whereas secretion of the anti-inflammatory cytokine IL-10 was induced.[34] Collectively, these properties of MSCs may be beneficial in COPD.

Antimicrobial effects

In addition to their anti-inflammatory effects, antimicrobial effects are also ascribed to MSCs.[35–37] These include direct inhibitory effects of MSCs on bacterial growth and indirect effects via secretion of immune-mediators that activate other (inflammatory) cells. Indeed, MSCs and MSC-derived conditioned medium directly reduce the growth rate and survival of several respiratory pathogens (*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumoniae*),[38] which was in part attributed to the secretion of antimicrobial peptides.[35 39–41] Indirect effects of MSCs on host defence may be mediated by secretion of immune-mediators such as IL-6, IL-8, granulocyte-macrophage colony-stimulating factor and macrophage migration inhibitory factor, which recruit neutrophils and enhance neutrophil antimicrobial activity.[42] Both direct and indirect effects of MSCs may thus contribute to host defence responses towards invading pathogens, which may be relevant for preventing or treating COPD exacerbations as these significantly contribute to morbidity and mortality in patients with COPD.

Lung epithelial and endothelial repair

In vitro models of lung epithelial and endothelial injury have demonstrated that MSCs can prevent injury and restore damaged monolayers. These models included scratch wound assays and electroporation of monolayers to assess effects of MSCs on wound closure and barrier function, or the addition of stimuli relevant in COPD pathogenesis, such as CSE, elastase, papain or pro-inflammatory cytokines or bacterial products such as lipopolysaccharide (LPS). Using these models, MSCs as well as MSC-conditioned medium (MSC-CM) were shown to induce repair and to protect against airway epithelial cell damage. MSCs and MSC-CM enhanced wound closure in scratch wounds in the A549 alveolar epithelial cell line and in primary small airway epithelial cells, possibly by increasing migration and proliferation of epithelial cells.[43 44] MSCs or unstimulated MSC-CM were also found to increase proliferation in lung epithelial cells injured by exposure to CSE or pro-inflammatory cytokines, suggesting a protective effect of MSCs.[45 46] Similar results were obtained in NCI-H292 airway epithelial cells[47] and in (human umbilical vein) endothelial cells,[48–50] including observations that adipose-tissue-derived stromal cell (AT-MSC) conditioned medium restored endothelial barrier function following CSE exposure.[49] Furthermore, MSCs reduced apoptosis in pulmonary cell cultures derived from papain-treated mice and in CSE-stimulated endothelial cells.[50 51] The potential mechanisms that underlie these effects are partially attributed to MSC-secreted factors: secretion of IL-6, IL-8 and chemokine (C-X-C motif) ligand 1 by MSCs was found to enhance A549 alveolar epithelial cell migration,[44] keratinocyte growth factor (KGF) secretion-induced epithelial cell proliferation[46] and reduction of the number of apoptotic cells was linked to vascular endothelial growth factor (VEGF)-A[50 51] and HGF.[52] It was furthermore suggested that MSCs support epithelial cell attachment and spreading via secretion of extracellular matrix proteins.[43 53] Finally, the observation that mitochondrial transfer from MSCs to airway epithelial cells may protect against cigarette smoke-induced injury is of special interest in view of the increasing number of reports on mitochondrial dysfunction in COPD.[54] This non-exhaustive list of factors constitutes only a small fraction of the factors secreted by MSCs[55] and future investigations are expected to further elucidate factors involved in MSC-mediated wound repair in vitro.

Preconditioning of MSCs

Preconditioning of MSCs, for example, with pro-inflammatory cytokines or hypoxic culture conditions was found to polarise MSCs towards an anti-inflammatory profile (referred to as MSC2) and to enhance their therapeutic potential in various disease models.[56] In line with this, preconditioning of MSCs had favourable effects on lung epithelial repair. Our own data show that preconditioning of MSCs with tumour necrosis factor (TNF)-α and IL-1β induced the expression of several growth factors and enhanced wound closure in NCI-H292 airway epithelial cells.[47] Similarly, TNF-α, IL-6 and IL-1β-stimulated MSCs induced A549 alveolar epithelial cell proliferation via increased KGF secretion.[46] Increased secretion of other growth factors, that is, VEGF, fibroblast growth factor 2, insulin-like growth factor 1 and HGF, in response to stimulation with TNF-α, LPS or hypoxia was also shown, but functional effects were not assessed.[57] Furthermore, mediators that are released by damaged alveolar epithelial cells increased the migration of MSCs and amniotic fluid-derived stem cells.[43 58] Overall, these data indicate that inflammatory mediators that are present in areas of tissue damage can alter the secretome of MSCs in such a way that it promotes wound repair. Theoretically, this inflammation-induced conditioning could enhance their effects in COPD, which is characterised by tissue damage and inflammation.[59]

In summary, in vitro studies show that MSCs exert a range of anti-inflammatory and immunomodulatory effects relevant to COPD, including improved protease/protease inhibitor balances, interactions with macrophages and antimicrobial effects. In addition, MSCs enhance wound healing in lung epithelial and endothelial cell models in vitro by increasing proliferation and migration and reducing apoptosis. The observation that MSC-CM exerts similar effects as MSCs supports the paracrine actions of MSCs, but many of the active factors still need to be elucidated. Furthermore, future investigations should focus on preconditioning of MSCs to enhance their regenerative and migratory potential.

EFFECTS OF MSCs ON COPD MODELS IN VIVO

The first animal study assessing the effects of MSC-based cell therapy in COPD showed promising results. Shigemura *et al* used porcine pancreatic elastase (PPE) to induce emphysema in rats, followed by intravenous administration of AT-MSCs (plastic adherent, $CD44^+/CD90^+/CD45^-$) on day 7. After 14 days, MSC treatment resulted in restoration of both alveolar and endothelial structures in AT-MSC-treated rats compared with control rats as shown by immunohistochemical analysis. A significant increase in proliferating cells and significantly lower numbers of apoptotic cells were observed in the treatment group. Additionally, improved gas exchange and exercise tolerance was observed.[60]

Following this initial encouraging observation, several studies have investigated in vivo effects of MSCs in experimental models of COPD and emphysema, mainly in rats and mice. A variety of protocols was used to induce COPD-like features (table 1), including instillation of proteolytic enzymes (PPE or papain) or chronic exposure to cigarette smoke with or without additional LPS. Administered MSCs were usually species-related allogeneic MSCs from the bone marrow or adipose tissue, but other sources of MSCs (amniotic fluid, lung or human) were also investigated. They were either administered systemically or locally via intratracheal instillation, with notable variation in frequency, dosage and timing of administration as well as in the period allowed to assess effects (see table 1 for details on study protocols). This variability complicates drawing generalisable conclusions on treatment regimens, although it is apparent that cell source and numbers, route of administration and timing of administration affect outcome.[52 61–64] Importantly, low doses of MSCs already improved lung architecture, and MSCs were still effective when administered as a late treatment in established emphysema, although there appeared to be a time-dependent decrease in effectiveness.[52]

The initial observation by Shigemura *et al*[60] showing that MSC-based cell therapy improves lung architecture, decreases apoptosis and increases cell proliferation was confirmed by several subsequent in vivo studies,[45 50 54 61 65-67] and a meta-analysis.[68] The exact mechanisms responsible for this repair have not yet been fully elucidated. The large body of circumstantial evidence is summarised in this section on in vivo COPD models, with a particular focus on effects of MSCs on inflammation and repair (see figure 1 for a schematic overview and table 1 for details on study protocols).

Anti-inflammatory effects

COPD is characterised by an enhanced inflammatory response,[29] and assessment of MSC-mediated effects on inflammation is therefore relevant. Assessment of the effect of MSC treatment on inflammation in in vivo studies mostly included immunohistochemical evaluation of pulmonary inflammatory infiltrates, bronchoalveolar lavage fluid (BALF) analysis of inflammatory cells and cytokines and analysis of mRNA expression of inflammatory cytokines in lung tissue.

MSC treatment reduced inflammatory cell infiltrates in peribronchiolar, perivascular and alveolar septa in lung tissue compared with control,[34 50 61 69] and a relative increase in alternatively activated (or M2) macrophages was observed.[34 61] This increased abundance of macrophages with an anti-inflammatory phenotype may contribute to reducing inflammation and enhancing repair responses.[70] One study showed no decrease in inflammatory parameters on MSC administration in an emphysema model of chronic LPS exposure.[71] In BALF, the total number of inflammatory cells and its subsets, that is, macrophages, neutrophils and lymphocytes, were lower in MSC-treated animals,[63 66 69] whereas there was a relative increase in type 2 macrophages.[34] BALF analysis of inflammatory cytokines involved in COPD pathogenesis showed a significant reduction of IL-1β, TNF-α and KC (murine IL-8 homologue) concentrations following MSC treatment,[32 63] although other studies did not observe such effects.[71 72] In line with decreased BALF-cytokine concentration, decreased mRNA expression of these cytokines were observed in emphysematous lung tissue following MSC treatment,[32 50 52 61 69] but results for monocyte chemoattractant protein 1 were conflicting.[50 69] Besides, treatment with MSCs decreased concentrations of several matrix metalloproteinases (MMPs), that is, MMP2, MMP9 and MMP12.[50] Although MMPs are important regulators of extracellular matrix homeostasis, abundance of MMPs has been linked to tissue destruction in emphysema[73] suggesting that decreased levels may contribute to tissue homeostasis.

Only limited in vivo data are available concerning potential mechanisms of action of MSCs. MSCs are thought to attenuate inflammation via reduction of *COX2* expression and PGE2 synthesis by macrophages[34] and decreased expression of TNF-α is attributed in part to an MSC-mediated increase in TGF-β secretion by macrophages.[69] It is furthermore hypothesised that induction of TGF-β signalling by MSCs inhibits MMP9 and MMP12 expression in alveolar macrophages.[50]

In conclusion, administration of MSCs appears to dampen inflammation in animal models of emphysema, reflected by a decrease in cytokine concentrations, inflammatory cells and infiltrates in lung tissue. There appears to be a role of MSC-mediated changes in macrophage polarisation towards alternatively activated type 2 macrophages, likely contributing to dampening of inflammation, but effects of MSCs on other immune cells were not systematically investigated. Furthermore, the precise mechanisms of action of MSCs in vivo are yet to be investigated.

Table 1 Animal models investigating MSCs in COPD: methods and main outcomes

Author (year)	Model	Cell source, number, route	Timing/frequency of cell therapy (from start)	Assessment of effects (from last cell therapy)	Main findings
Antunes *et al* [61] (2014)	C57BL/6 mice IT PPE weekly 4 weeks	AT-MSC, BM-MSC and LR-MSC 0.1×10^6 intravenous and intratracheal	Week 4 Once	7 days	All sources improved MLI, reduced inflammation and apoptosis. AT-MSC and BM-MSC improved mPAP and increased VEGF. Change of macrophages from M1 to M2 profile in BM-MSC group.
Gu, *et al* [34] (2015)	SD-rat CS exposure 12 weeks	BM-MSC 6×10^6 IT	Week 8–12, twice-weekly 10 times	28 days	Improved MLI and reduced inflammation (including increased M2 macrophages in BALF) through downregulation of COX2 and PGE2, possibly via alveolar macrophages.
Guan, *et al* [50] (2013)	SD-rat CS exposure 11 weeks	BM-MSC 6×10^6 IT	Week 7 Once	9 weeks	Improved MLI and PFT, reduction of pro-inflammatory mediators and proteases, reduced apoptosis. Increased VEGF, VEGFR and TGF-β.
Hoffman, *et al* [74] (2011)	C57BL6/J mice IT PPE once	BM-MSC and LR-MSC 0.5 and 1.0×10^6 IV (1), 0.33×10^6 IV (2)	Week 6 or 7, once (1) Twice-weekly, thrice (2)	22 (1) or 28 (2) days	Both sources improved MLI and increased IL-6 levels. No evidence of transdifferentiation. LR-MSC showed higher survival and retention in the lung compared with BM-MSCs.
Huh, *et al* [45] (2011)	Lewis rat CS exposure 6 months	BMC/BM-MSC 0.6×10^6/6×10^6 RB or MSC-CM	Month 6 Once	1, 7, 14, 28 days (BMC) and 8 weeks	Improved MLI and vascular parameters (mPAP, numbers of small pulmonary vessels), increased proliferation and reduced apoptosis. Paracrine effect rather than engraftment.
Ingenito, *et al* [53] (2012)	Sheep EB PPE monthly 5 months	Autol. LR-MSC $5–10\times10^6$ EB on scaffold	Week 8 Once	28 days	Increased tissue mass on CT with increased lung perfusion and ECM content. Only a fraction of LR-MSCs appeared to engraft. Proposed mechanism: promoted outgrowth of epithelial and endothelial cells through secretion of ECM components.
Katsha, *et al* [32] (2011)	C57BL/6 mice IT PPE once	BM-MSC 0.5×10^6 IT	Day 14 Once	7, 14 and 21 days	Improved MLI, increased levels of HGF, EGF and SLPI. Proposed mechanism via paracrine factors; infrequent engraftment or differentiation into epithelial cells.
Kennelly, *et al* [52] (2016)	NOD/SCID/IL-2Rγ^{null} mice IN PPE 6 times 2 weeks	BM-MSC (human) 0.5×10^6 IV or MSC-CM	Day 0 (1), 7 (2) or 12 (3) or day 0 (CM) Once	14 (1), 7 (2) or 16 (3) days or 14 days (CM)	Dose-dependent, protective effects of MSCs: decreased inflammation, less apoptosis and fibrosis. CM is protective but less effective. Proposed mechanism via HGF secretion.
Khedoe, *et al* [71] (2017)	APOE*3-Leiden mice IN LPS 2x/w 20 weeks	BM-MSC 0.5×10^6 IV	Week 14, 16, 18, 20 4 times	7 days	No effect on lung function parameters, MLI, lung tissue remodelling, pulmonary inflammatory infiltrates or cytokine levels in BAL or plasma.
Kim, *et al* [62] (2015)	C57BL/6J mice IT PPE once	UC-MSC (human) $0.01–0.1\times10^6$ IV	Day 7 Once	7 days	Dose finding: improved MLI and increased VEGF with 0.05×10^6 MSCs. No effects on apoptosis, MMPs, SLPI, TIMP1, HFG and FGF2.
Li, *et al* [54] (2014)	SD-rat CS exposure 56 days	BM-MSC and iPSC-MSC (human) 3×10^6 IV	Day 29 and 43 Twice	14 days	Both sources improved MLI, but iPSC-MSCs were more effective which is ascribed to higher mitochondrial transfer capacity of iPSC-MSCs.
Li, *et al* [65] (2014)	SD-rat CS exposure+LPS twice 12 weeks	AF-MSC 4×10^6 IT	Week 12 Once	20 and 40 days	Improved MLI, less apoptosis of AT2 cells, increased expression of SPA, SPC and TTF1. Proposed mechanism: integration into lung tissue and differentiation into AT2-like cells.
Liu, *et al* [66] (2015)	C57/B6 mice CS exposure 12 weeks	BM-MSC 4×10^6 IV	Week 5–12, once-weekly 8 times	14 days	Improved MLI, decreased apoptosis and inflammation, increased proliferation. No effects on PFT. Significant increase in numbers of BASCs.
Peron, *et al* [63] (2015)	C57BL/6 mice CS exposure 75 days +/-laser	T-MSC (human) 1×10^6 intranasal or intraperitoneal	Day 60 and 67 Twice	9 days	Laser-irradiated MSCs resulted in less inflammation, mucus production, collagen accumulation and tissue damage. Proposed mechanism: reduced NF-κB and NF-AT activation and increased IL-10.
Schweitzer, *et al* [49] (2011)	DBA/2J and C57BL/6 mice CS exposure 2 (1), 24 weeks (2) or VEGFR-inh (3)	AT-MSC (human) 0.3×10^6 IV	Day 14 once (1), month 2–4 twice-weekly, 4 times (2) or day 3 once (3)	1, 7, 21 days (1); 1 day (2) or 3 and 25 days (3)	Reduced inflammatory infiltration, decreased lung cell death and airspace enlargement. Effects on bone marrow and weight loss.
Shigemura, *et al* [60] (2006)	Lewis rat IT PPE once	AT-MSC 50×10^6 IV	Day 7 Once	7, 14, 21 and 28 days	Increased HGF. Inhibition of alveolar cell apoptosis, enhancement of epithelial cell proliferation and promotion of angiogenesis. Restored PFT.

Continued

Table 1 Continued

Author (year)	Model	Cell source, number, route	Timing/frequency of cell therapy (from start)	Assessment of effects (from last cell therapy)	Main findings
Song, *et al*[69] (2014)	SD-rat CS exposure 7 weeks	BM-MSC 6×10^6 IT	Week 8 Once	28 days	Less pro-inflammatory cytokines and inflammatory cells in BALF, improved histopathology and airflow obstruction. Proposed mechanism via induction of TGF-β1.
Tibboel, *et al*[64] (2014)	C57/BL6 mice IT PPE once	BM-MSC 0.5×10^6 IT (1) or 0.1×10^6 IV (2)	1 day prior, day 1 or day 21 (1); 30 min prior (2) once	19, 20 and 21 days	MSCs IV inhibited deterioration of lung function, without effects on histology. IT administration of MSCs had no effects.
Zhang, *et al*[72] (2014)	SD-rat CS exposure+IT LPS twice 8 weeks ±SPA (d 61) ±irr (d 90)	BM-MSC 4×10^6 IV	Day 90 Once	31 days	Following SPA suicide gene system infusion: increased recruitment of MSCs with induction of pulmonary fibrosis, proposed mechanism: due to vacant AT2 cell niches. Decreased IL-6 in BALF.
Zhen *et al*[67] (2008)	Lewis rat IT papain once +/-irr	BM-MSC 4×10^6 IV	Day 0 Once	28 days	Amelioration of emphysematous changes. MSC engraftment in recipient lungs and differentiation into AT2 cells. Suppression of alveolar cell apoptosis.
Zhen *et al*[51] (2010)	Lewis rat IT papain once	BM-MSC 4×10^6 IV	Day 0 (2 hours) Once	28 days	Improved MLI, restoration of reduced VEGFA expression.

AF, amniotic fluid; AT-MSC, adipose tissue-derived stromal cell; AT2, alveolar type 2 cell; Autol., autologous; BALF, bronchoalveolar lavage fluid; BASCs, bronchoalveolar stem cells; BM, bone marrow; BMC, bone marrow cells; COX2, cyclooxygenase 2; CS, cigarette smoke; EB, endobronchial; ECM, extracellular matrix; EGF, epidermal growth factor; HGF, hepatocyte growth factor; IL, interleukin; inh, inhibition; iPSC, induced pluripotent stem cell; irr, irradiation; LPS, lipopolysaccharide; LR, lung resident (lung-derived); MLI, mean linear intercept; MMPs, matrix metalloproteases; mPAP, mean pulmonary artery pressure; MSC, mesenchymal stromal cell; MSC-CM, MSC conditioned medium; NF-AT, nuclear factor of activated T-cells; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NOD/SCID/IL-2Rγ^{null}, non-obese diabetic/severe combined immunodeficiency IL-2 receptor gamma knockout; PFT, pulmonary function test; PGE2, prostaglandin E2; PPE, porcine pancreatic elastase; RB, retrobulbar; SD, Sprague Dawley; SLPI, secretory leucocyte protease inhibitor; SPA, surfactant protein A; SPC, surfactant protein C; T, tubal derived; TGF-β, transforming growth factor-β; TIMP, tissue inhibitor of metalloproteinases; TTF1, thyroid transcription factor 1; UC, umbilical cord; VEGFR, vascular endothelial growth factor receptor.

Lung tissue repair

Tissue destruction in emphysema is characterised by a loss of alveolar attachments, and MSC treatment was found to restore damaged alveolar structures in animal models of emphysema, reflected by a decrease in the mean linear intercept (a measure that describes the mean free distance in air spaces),[32 34 45 49–52 54 61 65–67 74] although this was not shown in chronic LPS-induced tissue destruction.[71] In some of these studies, MSCs were administered during induction of emphysema, suggesting that inhibition of emphysema development may have contributed to the effects (table 1). The observed restoration of damaged alveolar tissue is likely related to a decrease in numbers of apoptotic cells, usually assessed using TUNEL assays or by measuring caspase-3 concentrations[45 49 50 52 60 61 65-67] and to increased numbers of proliferating cells, that is, Ki67$^+$ or PCNA$^+$ cells.[45 60 66] Besides, an MSC-induced reduction in collagen deposition was observed in elastase-induced emphysema, suggesting antifibrotic effects that may contribute to inhibition of airway remodelling in COPD.[52] Factors that contribute to MSC-mediated tissue repair are described in the following section.

Paracrine effects

Administration of MSC-CM induced protective effects on lung tissue architecture,[45 52] in line with the concept that MSCs exert their effects in part via paracrine signalling, including secretion of growth factors. Indeed, following MSC administration, mRNA expression of HGF,[32 60] EGF,[32] VEGF[49–51 60] and KGF[45] was increased in emphysematous lung tissue compared with control. These growth factors are thought to contribute to restoration of tissue architecture in the lung,[75] and HGF in specific was linked to anti-apoptotic effects of MSCs.[52] The increased concentrations of HGF appeared to result from a combination of secretion by MSCs and induced secretion by local cells,[60] whereas for the other growth factors this was undetermined. Conflicting data were obtained for the effect of MSCs on TGF-β secretion.[50 61] However, the relevance and contribution of TGF-β in the context of COPD is unclear, as TGF-β has been linked both to small airway fibrosis in COPD[76] as well as to dampening of immune responses.[69]

Effects on endothelium

Endothelial integrity is essential for maintenance of the alveolar-capillary unit, with a pivotal role for VEGF signalling.[77] VEGF-receptor blocking can induce apoptosis of endothelial cells and emphysema, and treatment with human AT-MSCs can abrogate this effect.[49] Others have also demonstrated a lowering in destruction and apoptosis of endothelial cells following MSC treatment in cigarette or PPE-induced emphysema.[50 61] Functional effects include higher numbers of pulmonary capillaries corresponding with increased perfusion of the lung,[60] and reduced pulmonary artery pressure.[45 61]

Engraftment and transdifferentiation into lung structural cells

Engraftment and transdifferentiation of MSCs in epithelial cells have been proposed to contribute to the reconstruction of destructed lung architecture in emphysema. To address this, a number of animal studies have used green-fluorescent labelling of MSCs or administration of MSCs from male donors to female recipients, allowing detection of the Y-chromosome. MSCs were thus found to engraft into the lung tissue within 24 hours after administration, but their numbers appear to be low and decrease in a time-dependent fashion.[32 34 45 60] Although MSC engraftment and retention time in the lung can be increased following radiation[67 72] or by using lung-resident MSCs,[74] indications of functional benefits are lacking. Some studies provide evidence for transdifferentiation of MSCs into structural cells

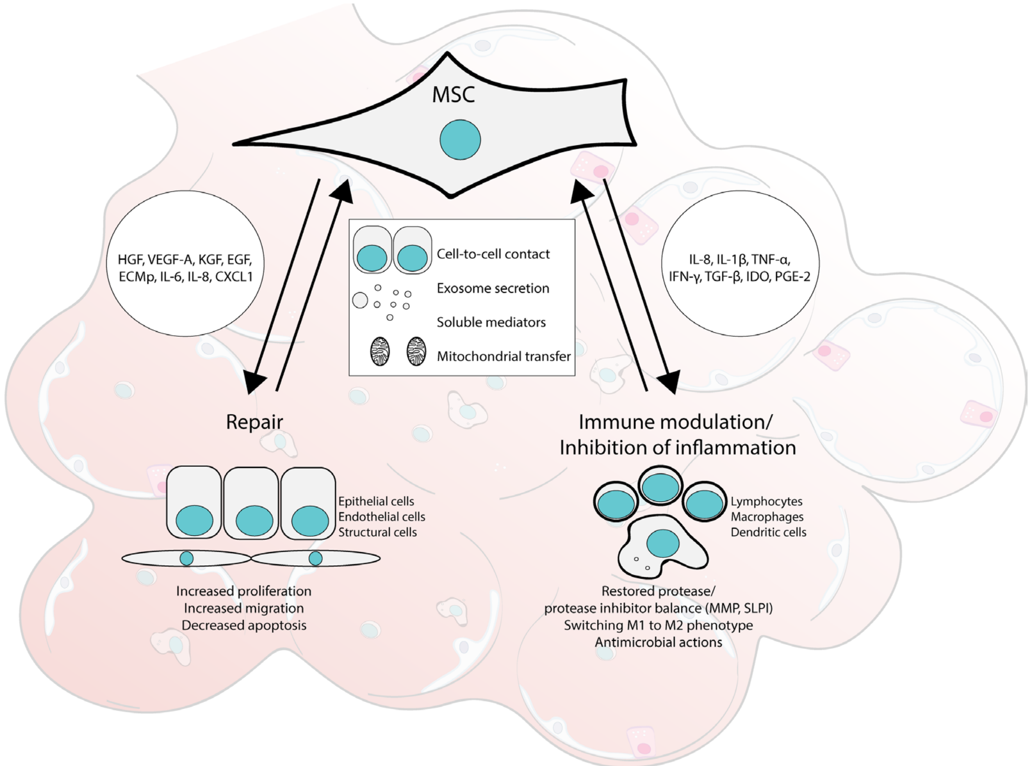

Figure 1 Mechanisms underlying the modulation of inflammation and lung tissue repair by MSCs in COPD. MSCs potentially act through cell-to-cell contact, mitochondrial transfer and secretion of soluble factors (either directly secreted or in exosomes), including growth factors, (anti)-inflammatory cytokines and chemokines (as indicated), thereby improving tissue homeostasis by favouring repair and dampening inflammatory responses. AMPs, antimicrobial peptides; CXCL1, chemokine (C-X-X motif) ligand 1; IDO, indolamine 2,3-dioxygenase; EGF, epidermal growth factor; ECMp, extracellular matrix proteins; GM-CSF, granulocyte macrophage colony-stimulating factor; HGF, hepatocyte growth factor; IFN-β, interferon-β; IL, interleukin; KGF, keratinocyte growth factor; MIF, migration inhibitory factor; MMP, matrix metalloproteinase; PGE2, prostaglandin E2; SLPI, secretory leucocyte protease inhibitor; TGF-β, transforming growth factor-β; TNF-α, tumour necrosis factor-α; VEGF-A, vascular endothelial growth factor-A.

of the alveolar unit,[65 67] but these data could not be reproduced by others.[32 74] The initial conclusions have been attributed to misinterpretation, and it should be concluded that evidence for direct contribution of MSCs to architectural reconstruction of destructed lung tissue is lacking.[78]

Collectively, these studies show that administration of MSCs restores lung architecture, decreases apoptosis and increases cell proliferation in animal models of emphysema. Several indicators of inflammatory responses are affected by MSCs, apparently in favour of dampening inflammation. Indirect evidence suggests that a regenerative environment is created via paracrine effects of MSCs and MSC-induced secretion of growth factors by local cells, resulting in higher concentrations of soluble factors that are relevant for tissue repair and that prevent apoptosis of endothelial cells. MSC engraftment and differentiation on the other hand are not considered to deliver a relevant contribution to tissue repair (figure 1). However, it should be taken into account that these studies were designed to detect maximum effects of MSCs, predominantly used 'acute' models of emphysema and were variable regarding cell numbers, route and timing of MSCs administered. These issues require further investigation, particularly in light of the fact that the clinical relevance of these preclinical results still needs to be established, as will be discussed in the 'Clinical trials' section.

CLINICAL TRIALS

The interest in using MSCs for the treatment of COPD or emphysema has translated into clinical trials. The first cell therapy study in COPD was an uncontrolled study in four subjects using autologous bone marrow mononuclear cells (BMMC) collected on treatment with granulocyte colony-stimulating factor.[79] In view of the design, small size and lack of statistical analysis, no conclusions can be drawn as to the efficacy of this treatment (despite reported changes in lung function and quality of life). Clearly the BMMC preparation used may have contained small numbers of MSCs, but this study cannot be viewed as an MSC intervention study. Since this review is focused on MSCs, cell therapy studies using bone marrow cells that were not further cultured and/or selected before administration are not further discussed. This section describes the main observations from clinical MSC trials, including an overview of ongoing trials (table 2). For more details, we refer to a recently published review on this topic.[80]

The first trial using MSCs in patients with moderate-to-severe COPD (GOLD II-III) was conducted by Weiss *et al:* the safety and efficacy of treatment with four intravenous infusions of allogeneic bone marrow-derived MSCs (BM-MSCs) from a pool of non-HLA-matched donors *(Prochymal)* was compared with placebo in 62 patients, in a double-blind study. Infusions (100×10^6 cells/infusion) were well tolerated in all patients, and no clinically relevant adverse events related to the cell therapy were reported. Treatment with MSCs had no effect on clinical parameters, including pulmonary function and quality of life. There was a significant decrease in C reactive protein (CRP) levels up to 1 month after the first infusion. In the discussion, the authors suggest that effects of MSCs may have been missed due to the dosage and treatment regimen, sample size or due to

Table 2 Clinical trials investigating MSCs for COPD treatment

NCT number	Study design	No.	Cell type	Route	FU	Primary outcome	Study completion	Remarks
NCT00683722 (USA)[81]	Placebo-ctrl Randomised Double-blind	62	Allog. BM-MSC	Intravenous	2 y	Safety/efficacy (phase II)	2010 December	
NCT01306513 (The Netherlands)[82]	Single group Open label	10	Autol. BM-MSC	Intravenous	1 y	Safety (phase I)	2012 November	With LVRS
NCT01758055 (Iran)[90]	Single group Open label	12	Autol. BM-MSC	Endobronchial	n.s.	Safety (phase I)	2014 January	
NCT01872624 (Brazil)[83]	Placebo-ctrl Non-randomised Open label	10	Allog. BM-MSC	Endobronchial	4 mo	Safety (phase I)	2015 March	With EB valves
NCT02645305 (Vietnam)[90]	Single group Open label	20	Autol. AT-MSC	Intravenous	1 y	Safety/efficacy (phase II)	2016 December	With APRP
NCT02041000 (USA)[90]	Single group Open label	100	Autol. AT-MSC	Intravenous	6 mo	Safety/efficacy (phase II)	2017 January	Commercial (Bioheart)
NCT02412332 (Brazil)[90]	Placebo-ctrl Randomised Open label	20	Autol. AT-MSC, BMMC or both	Intravenous	1 y	Safety/efficacy (phase II)	2017 April	
NCT01849159 (Russia)[90]	Placebo-ctrl Randomised Open label	30	Allog. BM-MSC	Intravenous	2 y	Safety/efficacy (phase II)	2017 June	Hypoxia-cultured
NCT02216630 (USA)[90]	Single group Open label	200	Autol. AT-MSC	Intravenous	1 y	Safety/efficacy (phase II)	2017 August	Commercial (Kimera)
NCT02161744 (USA)[90]	Single group Open label	60	Autol. AT-MSC	Intravenous	1 y	Safety/efficacy (phase I)	2017 August	
NCT01559051 (USA)[90]	Single group Open label	100	Autol. AT-MSC	Intravenous/ endobronchial	6 mo	Safety/efficacy (phase II)	2017 November	Commercial (Ageless Regenerative Institute)
NCT02348060 (USA)[90]	Single group Open label	75	Autol. AT-MSC	n.s.	1 y	Quality of life	2018 February	Commercial (StemGenex)

Allog., allogeneic; APRP, activated platelet-rich plasma (from peripheral blood); AT-MSC, adipose tissue-derived stem cells; Autol., autologous; BM-MSC, bone marrow-derived mesenchymal stromal cells; BMMCs, bone marrow-derived mononuclear cells; Ctrl, controlled; d, day; FU, follow-up; LVRS, lung volume reduction surgery; mo, month; n.s., not specified; NCT, ClinicalTrials.gov Identifier number; No., number of participants enrolled; route, route of administration; y, year.

the chronic nature of COPD, which might therefore be a less effective target for MSCs compared with more acute inflammatory disorders, such as ARDS.[81]

The next clinical trial that investigated the safety of MSC administration in patients with severe-to-very severe COPD (GOLD III-VI) was conducted by our own group. The study protocol was designed around patients who were eligible for bilateral lung volume reduction surgery. Autologous MSCs ($1–2\times10^6$ cells per kg bodyweight) were administered twice intravenously in between the two surgical interventions, which thus allowed comparison of lung tissue obtained before and after MSC administration. Seven patients completed the study protocol, without occurrence of therapy-related adverse events. Changes in FEV_1 and bodyweight were attributed to the surgical intervention. The majority of analysed tissue parameters were unchanged in post-MSC tissue, except for increased CD3, CD4 (T-cell markers) and CD31 (endothelial cell marker) expression. Although we cannot formally exclude surgery-related effects underlying these changes, as a control group is lacking, the observed increase in CD31 may be indicative of a reparative response. The increase in the endothelial marker CD31 is especially relevant in view of the observation that loss of endothelial integrity contributes to development of emphysema.[82]

Finally, a clinical trial designed to assess MSC effects on local inflammation resulting from endobronchial valve (EBV) placement for severe-to-very severe COPD (GOLD III-IV) demonstrated the safety of endobronchial instillation of allogeneic BM-MSCs (100×10^6 cells) prior to EBV placement, compared with saline-treated controls (five patients per group). In the MSC treatment group, serum CRP concentrations significantly improved up to 90 days follow-up.[83] However, given the study design, this study cannot be viewed as one focused on MSC therapy for COPD.

At present, several trials evaluating cell therapy for the treatment of COPD are still ongoing or their results are awaited (table 2). In view of the outcomes of the conducted clinical trials, it seems reasonable to optimise treatment protocols and identify relevant measurable outcome parameters for future clinical trials. However, the majority of these comprise (commercially initiated) safety trials, designed as open-label studies lacking a control group and will therefore likely add limited information. Besides, it needs to be noted that most of these trials have not been reviewed or approved by relevant regulatory agencies and therefore caution is warranted when interpreting the data obtained from these trials. Apart from these registered trials, stem cell clinics in several countries offer unproven stem cell treatments with a variety of cells, including MSCs. To protect patients, the ATS RCMB Stem Cell working group has called for intensification of communication and collaboration between patients, scientists and respiratory disease societies worldwide to improve patient education, research and effective legislation.[84]

FUTURE DIRECTIONS

There are several possible explanations for the lack of translation of the promising preclinical data of MSC treatment to clinically relevant effects in patients with COPD. The animal models were optimised to detect maximum effects, and used higher cell numbers per kilogram bodyweight and more 'acute' models of COPD or COPD-like inflammation which can enhance MSC efficacy. The available preclinical in vivo studies used invasive readouts for analysis, such as tissue resection and BALF, contrary to most clinical trials that investigated effects on minimally invasive clinical parameters, such as pulmonary function testing or quality of life assessment. Although relevant, these clinical read-outs might not be responsive to MSC therapy on short-term treatment. It is therefore important to consider parameters that might change before clinical improvement. These may include induction of CD31 expression in lung tissue, as indicated by the data from our own institution,[82] or alterations in the composition of inflammatory cells in sputum, BALF and lung tissue. Likewise, timing, duration, preconditioning, dosage and frequency of administration as well as the route of administration need to be optimised in humans. Although it can be argued that animal studies used higher doses of MSCs than human studies, thus explaining the lack of clinical efficacy in COPD, human clinical studies in osteoarthritis and Crohn's disease suggest that the highest dose may not always provide the best results.[85 86] These findings underscore the importance of dose finding in future clinical studies. Further research should clarify whether route of administration influences the potential of MSCs, and whether different routes should be used when aiming to target airway disease versus emphysema in COPD. Furthermore, an inflammatory environment appears to increase the potential of MSCs in vitro. This may have important implications for future research on the use of MSCs in the treatment of COPD. First, as COPD is a chronic inflammatory disease, MSCs might be less effective in COPD compared with more acute inflammatory conditions such as ARDS. This might limit the therapeutic potential of MSCs in stable COPD or have consequences for dosage and/or frequency of MSC administration. Besides, future studies should address the possibility that MSCs could be more effective in subgroups of patients with COPD with higher levels of inflammatory markers or during active inflammation (eg, during exacerbations). Second, this suggests that studies are needed to investigate the effect of preconditioning of MSCs with pro-inflammatory cytokines to improve their therapeutic potential in clinical COPD trials. In line with this, administration of MSCs engineered to overexpress mediators that increase their therapeutic potential, as for instance shown for MSCs overexpressing angiopoietin-1 or IL-10 which prevent ARDS in mice,[87 88] may hold promise for future applications. Finally, there is some evidence linking heterogeneity of MSCs to efficacy in vivo, and further studies are needed to identify 'superior' cell products to enhance the clinical efficacy of MSCs.[89]

It is evident that despite the encouraging preclinical data, a cure for COPD based on administration of MSCs is not yet at hand. It will take time and effort to elucidate the precise mode of action of MSCs. This may result in identification of biomarkers in patients with COPD that can serve as an early indicator that the progressive course of COPD is amended, which is essential to optimise treatment protocols. To reduce costs and limit the number of patients required to answer the unresolved questions, there is an urgent need for preclinical models that accurately reflect the human pathophysiology, for example, ex vivo lung perfusion, organoids, microfluidic lung-on-a-chip and lung tissue slices.

CONCLUSION

Preclinical studies suggest that cell therapy using MSCs is a potential new treatment strategy for COPD. Both in vitro and in vivo studies have demonstrated the regenerative potential of MSCs, which is reflected by their ability to induce airway epithelial and endothelial repair, and restore lung tissue architecture in emphysematous lungs in animal models. These effects relate to increased proliferation and migration of target cells and reduction of apoptosis. Besides, MSCs modulate immune responses, dampen inflammatory responses in preclinical COPD models and affect protease/protease inhibitor balances favouring tissue homeostasis. The precise mechanisms are not fully unravelled, although the involvement of a number of secreted factors including cytokines and growth factors has been suggested. Whereas initial studies in a limited number of patients with COPD have revealed that MSC treatment is safe, so far there is no evidence for clinically relevant effects and further studies are needed to demonstrate that MSC-based treatments are of clinical relevance to patients with COPD. Important challenges need to be addressed, including optimising the MSC treatment regimens and identification of responsive outcome parameters, for example, in lung tissue. Such information may guide us in the choice of clinical outcome parameters for MSC treatment in patients with COPD. The lack of effective interventions to restore lung function in COPD will be an important driver for these and other innovative approaches to the treatment of this highly prevalent disease.

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