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Mesenchymal stem cells: mechanisms of potential therapeutic benefit in ARDS and sepsis

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Multipotent mesenchymal stem (stromal) cells (MSCs) have shown promising therapeutic effects in preclinical models of both acute respiratory distress syndrome (ARDS) and sepsis. Although initial research focused on the ability of MSCs to engraft at sites of tissue injury, increasing evidence suggests that MSCs have their therapeutic effects through mechanisms unrelated to long-term incorporation into host tissue. One of the most compelling of these pathways is the ability of MSCs to interact with injured tissue through the release of soluble bioactive factors. This Review provides an overview of the general properties of MSCs, and then outlines ways in which the paracrine effects of MSCs might reduce lung injury and enhance lung repair in ARDS and sepsis. Finally, we summarise ongoing challenges in MSC research and identify areas in which the discipline might progress in the coming years.

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Introduction

Advances in supportive care have markedly improved survival for patients with acute respiratory syndrome (ARDS)[1] and sepsis.[2] However, both syndromes continue to be associated with high mortality and morbidity.[3,4] Despite decades of clinical trials, effective pharmacotherapy for either syndrome remains elusive.[5,6]

A growing body of evidence suggests that cell-based therapy with stem or progenitor cells holds substantial therapeutic promise for a host of inflammatory disorders, including ARDS and sepsis.[7,8] Although several cell types, including endothelial progenitor cells and embryonic stem cells, are under investigation, this Review will focus on multipotent mesenchymal stem (or stromal) cells (MSCs).

We summarise the general properties of MSCs, explore how the paracrine effects of MSCs might affect ARDS and sepsis pathobiology, and review ongoing challenges in translational MSC research. We therefore provide a clinician-oriented framework for understanding of the expanding scientific literature for MSCs and how this research might eventually affect clinical care.

MSCs

Overview

Originally isolated from bone marrow and termed fibroblastic colony-forming units,[9] MSCs are non-haemopoietic stromal cells that have the ability to adhere to plastic in standard tissue culture, express characteristic cell-surface markers, and differentiate in vitro to osteoblasts, adipocytes, and chondroblasts.[10] MSCs can be isolated from most types of mesenchymal tissue, such as bone marrow, umbilical cord blood, placenta, and adipose tissue.[11]

MSCs have several properties that make them attractive therapeutic candidates for treatment of acute disease. They are regarded as non-immunogenic because of their low constitutive expression of major histocompatibility complex (MHC) type I and the absence of both MHC type II and T-cell co-stimulatory molecules. This property theoretically allows for allogeneic transplantation without the need for HLA matching or immunosuppression.[11] Unlike embryonic stem cells, MSCs have low tumorigenicity and a short lifespan in vivo.[12] Finally, once isolated from host tissue, MSCs can be expanded rapidly ex vivo, which enables prompt clinical administration.[13] In view of these advantages, MSCs have become an active focus of investigation for a wide range of diseases, such as ischaemic cardiomyopathy,[14] chronic obstructive pulmonary disease,[15] acute neurological injuries,[16] graft-versus-host disease,[17] sepsis, and acute lung injury.

Mechanisms of potential benefit

Understanding of the mechanisms by which MSCs promote tissue repair continues to progress. MSCs were initially thought to provide a niche for haemopoietic cells with their similarities to bone marrow stroma and ability to serve as feeder layers for haemopoietic cells in culture.[18] Initial research also focused on the ability of

Key messages

- Despite advances in supportive care and decades of clinical trials, acute respiratory distress syndrome (ARDS) and sepsis remain associated with significant morbidity and mortality.
- A growing body of literature suggests that multipotent mesenchymal stem cells (MSCs) hold significant therapeutic promise for ARDS and sepsis.
- Although early research focused on the ability of MSCs to engraft at the site of tissue injury, newer evidence suggests that MSCs interact with host tissue partly through the release of soluble paracrine factors. These paracrine effects might modulate important pathobiological pathways in ARDS and sepsis.
- MSCs have been shown to have anti-inflammatory effects on host tissue in preclinical models of ARDS and sepsis. Potential anti-inflammatory paracrine factors include IL-1ra, TSG-6, IGF1, and prostaglandin E2.
- MSCs have been shown to preserve both vascular endothelial and alveolar epithelial barrier function in preclinical models of ARDS and sepsis.
- Preclinical models suggest that MSCs improve alveolar fluid clearance, partly through the release of FGF7.
- MSCs have been reported to have antimicrobial effects, partly by increasing the phagocytic activity of host immune cells. These effects might be mediated by the release of lipocalin-2 and LL-37. MSCs might also prevent apoptosis of host cells, although this effect is not well understood.
- Experimental studies and ongoing clinical trials will both have important roles in the addressing of current gaps in knowledge.

MSCs to structurally engraft at the site of tissue injury.[19–21] However, with refined research techniques, MSC engraftment seems to be a rare event[22,23] of unclear physiological significance.[13,24]

A growing number of studies have shown that MSCs have immunomodulatory and anti-inflammatory effects despite minimum or absent engraftment.[25–28] Consequently, research has shifted towards identification of alternative pathways through which MSCs interact with host tissue, including interactions between cells, direct interactions with the host immune system, and mitochondrial transfer. The pathway with the most robust supporting evidence is the ability of MSCs to coordinate tissue repair through the release of soluble paracrine factors.[29]

This Review focuses on the paracrine effects of MSCs that modulate important pathobiological pathways in ARDS and sepsis, including inflammation, endothelial and epithelial cell injury, alveolar fluid clearance, antimicrobial activity, and apoptosis (figure). A summary of referenced literature is included in tables 1–4.

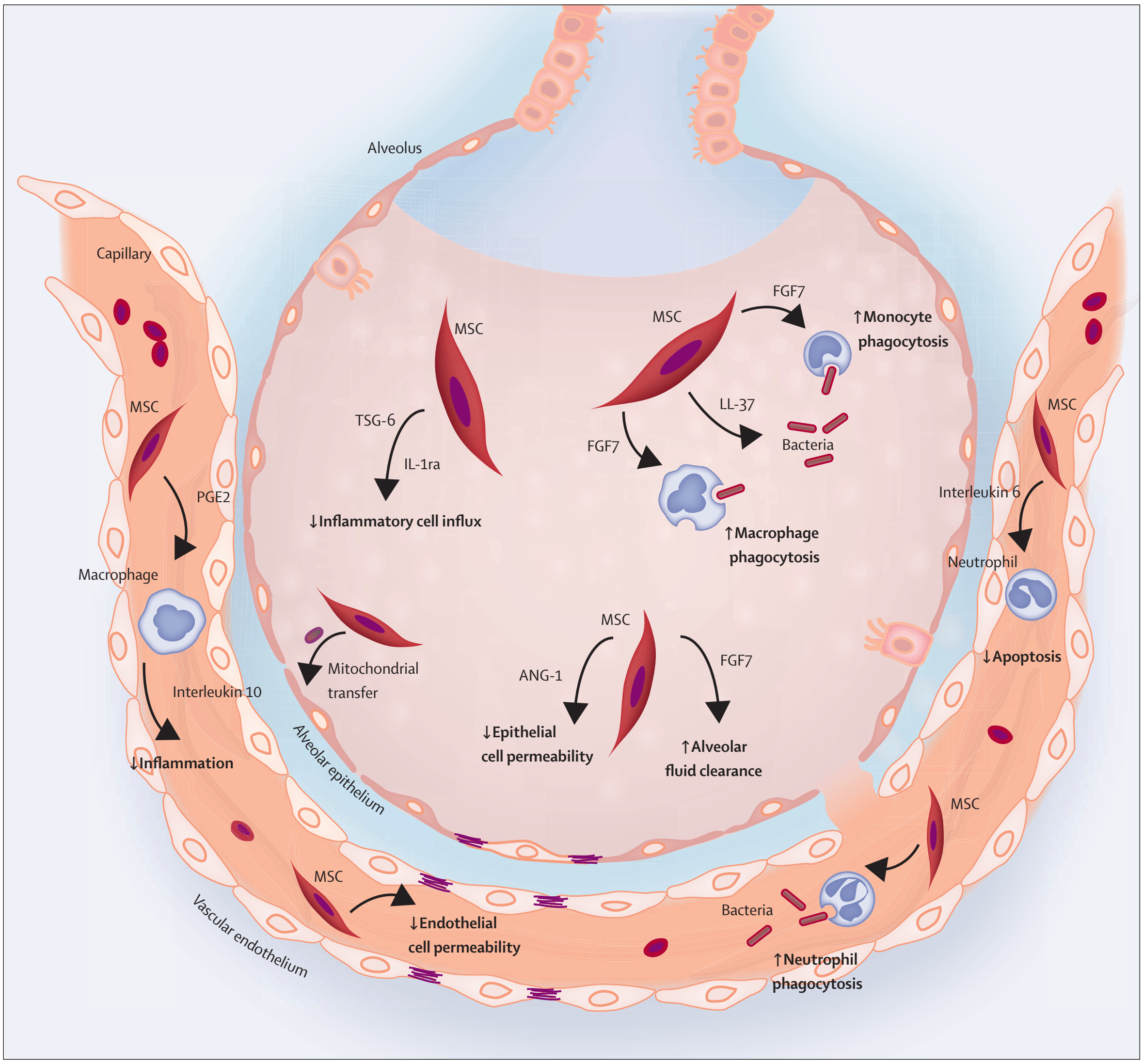

Figure: Potential therapeutic effects of MSC therapy in ARDS and sepsis

Protein-rich oedema fluid and inflammatory cells fill an injured alveolus as a result of a bacterial infection. MSCs have been shown in many preclinical studies to modify important pathobiological pathways in ARDS and sepsis through the release of paracrine factors. These modulatory effects include: exertion of anti-inflammatory effects on host tissue; reduction of the permeability of the alveolar epithelium and vascular endothelium; improvement of alveolar fluid clearance; improvement of the phagocytic activity of macrophages, monocytes, and neutrophils; and exertion of anti-apoptotic effects on host cells, although this pathway is not well characterised. Finally, MSCs might modulate tissue repair through direct mitochondrial transfer with host cells. How the route of MSC delivery affects the interaction between MSCs and host tissue is not well understood. Pathways depicted in the capillary and alveolus are not necessarily exclusive to that anatomical compartment, nor are they dependent on a certain route of MSC delivery. MSC=mesenchymal stem (stromal) cell. ARDS=acute respiratory distress syndrome. PGE2=prostaglandin E2.

Paracrine pathways

Anti-inflammatory effects

Disordered inflammation has a central role in the pathogenesis of ARDS and sepsis.[53,54] Substantial evidence from models of both lung injury and sepsis suggests that MSCs have an anti-inflammatory effect on host tissue, partly through the release of paracrine factors.

Preclinical acute lung injury models

The anti-inflammatory effects of MSCs have been reported in several models of acute lung injury. In a bleomycin lung injury model, intravenous MSCs delivered 6 h after injury normalised levels of proinflammatory cytokines when measured on day 14.[25] A paracrine mechanism was suggested by the small number of donor-derived cells that localised to the injured lung. Similarly, intratracheal delivery of MSCs reduced concentrations of proinflammatory cytokines and numbers of total cells and neutrophils in bronchoalveolar lavage (BAL) fluid after injury with lipopolysaccharide (LPS), despite low levels of engraftment.[27,30] Finally, treatment with MSC-conditioned media rather than actual MSCs has been noted to decrease BAL concentrations of cytokines and inflammatory cells in ventilator-induced lung injury models in rats, which supports the presence of soluble anti-inflammatory factors.[31,32]

Several anti-inflammatory factors secreted by MSCs have been identified. A subpopulation of MSCs produce

	Injury model	MSC source	MSC delivery method	Major finding	Evidence for specific paracrine factors
Rojas et al[25]	Murine bleomcyin	MBMDMSC	Intravenous 6 h after injury	↓Proinflammatory cytokines	NA
Gupta et al[27]	Murine intratracheal endotoxin	MBMDMSC	Intratracheal 4 h after injury	↑Survival ↓BAL markers of inflammation	NA
Mei et al[30]	Murine intratracheal LPS	MBMDMSC	Intravenous 30 min after injury	↓BAL markers of inflammation	NA
Curley et al[31]	Rat VILI	RBMDMSC	Intratracheal or intravenous 15–30 min after injury	↓BAL proinflammatory cytokines Similar results with MSC-M	NA
Curley et al[32]	Rat VILI	RBMDMSC	Intravenous immediately and 24 h after injury	↓BAL inflammatory cells and proinflammatory cytokines Similar results with MSC-M	NA
Ortiz et al[33]	Murine bleomycin	MBMDMSC	Intravenous after injury	↓BAL neutrophils and TNF	Anti-inflammatory effects of MSC-M in vitro dependent on IL-1ra
Danchuk et al[34]	Murine OA LPS	HBMDMSC	OA 4 h after injury	↓BAL inflammatory cells and proinflammatory cytokines	Blockage of TSG-6 synthesis by MSCs attenuates anti-inflammatory effects
Ionescu et al[35]	Murine intratracheal LPS	MBMDMSC	Intratracheal 4 h after injury	↓BAL inflammatory cells and improved lung histology with both MSCs and MSC-M MSC-M induce M2 anti-inflammatory phenotype in vitro and in vivo	Recombinant IGF1 partly reproduces in vitro and in vivo anti-inflammatory effects of MSC-M
Xu et al[28]	Murine intraperitoneal LPS	MBMDMSC	Intravenous 1 h after injury	↓Plasma proinflammatory cytokines Improved lung histology	NA
Weil et al[36]	Rat intravenous LPS	RBMDMSC	Intravenous 1 h after injury	↓Plasma and myocardial levels of proinflammatory cytokines Improved cardiac function	NA
Luo et al[37]	Murine CLP	MBMDMSC	Intravenous 3 h after injury	↑Survival ↓Renal and plasma expression of proinflammatory cytokines	NA
Mei et al[38]	Murine CLP	MBMDMSC	Intravenous 6 h after injury	↑Survival ↓Plasma proinflammatory cytokines ↑Organ function ↓Alveolar inflammatory cells and proinflammatory cytokines	NA
Choi et al[39]	Murine peritonitis	HBMDMSC	Intraperitoneal 15 min after injury	↓Intraperitoneal inflammatory cell infiltrate	Anti-inflammatory effects in vitro and in vivo dependent on TSG-6
Németh et al[40]	Murine CLP	MBMDMSC	Intravenous 24 h before, during, or 1 h after injury	↑Survival ↓Plasma proinflammatory cytokines ↑Organ function	MSCs produce PGE2 which induces an M2 phenotype, increasing macrophage production of interleukin 10
Lee et al[41]	Ex-vivo perfused human lung directly injured with *Escherichia coli*	HBMDMSC	Intravenous or intrabroncheal 1 h after injury	↓Neutrophil influx	NA

MSC=mesenchymal stem (stromal) cell. AB=antibody. BAL=bronchoalveolar lavage. CLP=caecal ligation and puncture. HBMDMSC=human bone marrow-derived MSCs. LPS=lipopolysaccharide. MBMDMSC=murine bone marrow-derived MSCs. MSC-M=MSC-conditioned media. OA=oral aspiration. PGE2=prostaglandin E2. RBMDMSC=rat bone marrow-derived MSCs. TNF=tumour necrosis factor. VILI=ventilator-induced lung injury. NA=not applicable.

Table 1: **Summary of the scientific literature lending support to the anti-inflammatory effects of MSCs**

Study	Injury model	MSC source	MSC delivery method	Major finding	Evidence for specific paracrine factors
Regulation of endothelial permeability					
Pati et al[42]	Human vascular endothelial cells in vitro	HBMDMSC	Co-culture	↓Paracellular permeability	NA
Pati et al[43]	Rat haemorrhagic shock	HBMDMSC	Intravenous at 1 and 24 h after injury	Stabilisation of endothelial cells	NA
Németh et al[40]	Murine CLP	MBMDMSC	Intravenous 24 h before, during, or 1 h after injury	↓Vascular permeability	NA
Lee et al[44]	Ex-vivo perfused human lung injured with endotoxin	HBMDMSC	Intrabroncheal 1 h after injury	Restoration of endothelial permeability to control levels Reproduced with MSC-M	NA
Lee et al[41]	Ex-vivo perfused human lung directly injured with *Escherichia coli*	HBMDMSC	Intravenous or intrabronchеal 1 h following injury	Restoration of alveolar fluid clearance	NA
Regulation of epithelial permeability					
Fang et al[45]	Human ATII cells in vitro	HBMDMSC	Co-culture	Normalisation of epithelial cell protein permeability	↑ANG-1 measured in co-culture ANG-1 knockout MSCs without therapeutic effect
Goolaerts et al[46]	Rat alveolar epithelial cells in vitro	HBMDMSC	Co-culture	Normalisation of epithelial cell protein permeability	↑IL-1ra, ↑PGE2, in MSC-M conditioned with inflammatory and hypoxic stimuli

MSC=mesenchymal stem (stromal) cell. ATII=alveolar epithelial type II cells. CLP=caecal ligation and puncture. HBMDMSC=human bone marrow-derived MSCs. MBMDMSC=murine bone marrow-derived MSCs. MSC-M=MSC-conditioned media. PGE2=prostaglandin E2. NA=not applicable.

Table 2: **Summary of the scientific literature lending support to the ability of MSCs to regulate endothelial and epithelial permeability**

	Injury model	MSC source	MSC delivery method	Major finding	Evidence for specific paracrine factors
Goolaerts et al[46]	Rat alveolar epithelial cells in vitro	HBMDMSC	Co-culture	↑Transepithelial Na transport ↑Apical expression of αENaC with MSC-M	Effects not seen with FGF7-depleted media
Lee et al[44]	Ex-vivo perfused human lung injured with endotoxin	HBMDMSC	Intrabronchеal 1 h after injury	↓Lung water Normalisation of alveolar fluid clearance Preservation of net fluid transport Partial restoration of apical αENaC expression in vitro	FGF7-depleted media with minimal effect on fluid clearance Addition of recombinant FGF7 to media restores activity Blockage of FGF7 expression prevents therapeutic effect in vitro
McAuley et al[47]	Ex-vivo perfused human lungs rejected for transplant	HBMDMSC	Added to perfusate	Normalisation of alveolar fluid clearance	Pre-treatment with FGF7-blocking AB reduces effect

MSC=mesenchymal stem (stromal) cell. AB=antibody. HBMDMSC=human bone marrow-derived MSCs. MSC-M=MSC-conditioned media.

Table 3: **Summary of the scientific literature lending support to the ability of MSCs to improve alveolar fluid clearance**

IL-1ra, which inhibits cytokine stimulation of a helper-T-lymphocyte line and suppresses macrophage production of the inflammatory cytokine tumour necrosis factor α (TNFα) in an IL-1ra-dependent manner.[33]

TSG-6, a potent anti-inflammatory protein, has also been identified as a potential paracrine factor. In a murine model of lung injury using LPS, MSCs upregulated expression of TSG-6, while decreasing cytokine levels and inflammatory cell counts in BAL fluid.[34] Knockdown of TSG-6 expression in MSCs nullified most of these anti-inflammatory effects when MSCs were given after injury. In support of these findings, other studies show that intravenous administration of TSG-6 reduced alveolar concentrations of proinflammatory cytokines and improved survival in a bleomycin lung injury model.[55] TSG-6 also mediated the ability of MSCs to decrease infarct size and improve cardiac function after myocardial infarction in mice.[56]

Finally, evidence suggests that IGF1 might have an important role in mediation of the anti-inflammatory effects of MSCs. Ionescu and colleagues[35] reported that MSC-conditioned media restricted the alveolar influx of inflammatory cells and improved the histological appearance of the lung when given after intratracheal LPS injury in an in-vivo mouse model of lung injury. MSC-conditioned media was also shown to promote differentiation of alveolar macrophages to an M2 anti-inflammatory phenotype both in vitro and in vivo.[35] These anti-inflammatory effects were partly reproduced in vitro and in vivo by the delivery of recombinant IGF1.[35]

Preclinical sepsis models

MSCs have also been shown to have anti-inflammatory effects in several preclinical models of sepsis. Intravenous MSCs reduce plasma concentrations of inflammatory cytokines after intraperitoneal LPS,[28]

	Injury model	MSC source	MSC delivery method	Major finding	Evidence for specific paracrine factors
Antimicrobial effects					
Krasnodembskaya et al[48]	Murine peritonitis	HBMDMSC	Intravenous 1 h after injury	↑Survival ↓Circulating bacteria ↑Phagocytic activity of mononuclear cells	NA
Hall et al[49]	Murine CLP			↑Survival ↓Organ injury ↑Neutrophil phagocytosis ↓Circulating bacteria	NA
Krasnodembskaya et al[50]	Murine intratracheal *Escherichia coli*	HBMDMSC	Intratracheal 4 h after injury	MSCs and MSC-M inhibit bacterial growth in vitro ↓Lung bacterial load	MSCs able to increase LL-37 production in vitro Blockage of LL-37 synthesis prevents antimicrobial effects in vivo
Mei et al[38]	Murine CLP	MBMDMSC	Intravenous 6 h after injury	↓Bacterial burden in spleen ↑Phagocytic activity of ITGAM-positive cells	NA
Luo et al[37]	Murine CLP	MBMDMSC	Intravenous 3 h after injury	↓Circulating bacteria	NA
Lee et al[41]	Ex-vivo perfused human lung injured with intrabroncheal *E coli*	HBMDMSC	Intravenous or intrabroncheal 1 h after injury	↑Alveolar macrophage phagocytosis ↓Alveolar bacterial burden	Intrabroncheal FGF7 ↓alveolar bacterial load and ↑alveolar macrophage phagocytosis In vitro, FGF7-positive monocytes increase bacterial killing and monocyte survival FGF7-blocking AB nullifies antimicrobial effects ex vivo and in vitro
Gupta et al[51]	Murine intratracheal *E coli*	MBMDMSC	Intratracheal 4 h after injury	↑Survival ↑Alveolar bacterial clearance	↑Lipocalin-2 in BAL fluid Lipocalin-2 AB blocks antimicrobial effects of MSCs
Anti-apoptosis					
Raffaghello et al[52]	Human neutrophils in vitro	HBMDMSC	Co-culture	↓Apoptosis of resting and activated neutrophils with both MSCs and MSC-M	Effect mediated partly by interleukin 6

MSC=mesenchymal stem (stromal) cell. AB=antibody. BAL=bronchoalveolar lavage. CLP=caecal ligation and puncture. HBMDMSC=human bone marrow-derived MSCs. MBMDMSC=murine bone marrow-derived MSCs. MSC-M=MSC-conditioned media. NA=not applicable.

Table 4: **Summary of the literature supporting the antimicrobial and anti-apoptotic effects of MSCs**

intravenous LPS,[36] and ligation and puncture of the caecum;[37,38] all despite limited or absent MSC engraftment. MSCs also seem to attenuate end-organ inflammatory damage.[40] Intravenous MSCs improve lung histology and decrease concentrations of pro-inflammatory cytokines in BAL fluid after infection,[28,38] decrease concentrations of inflammatory cytokines in cardiac tissue and improve cardiac function after intravenous LPS,[36] and also lower renal expression of proinflammatory cytokines and improve serological markers of kidney function after caecal ligation and puncture.[37] These effects occurred without substantial MSC localisation to the studied tissue, which suggests a paracrine mechanism.

As with lung injury models, investigators have used infection models to identify paracrine factors that might contribute to the observed benefits of MSCs. Recombinant TSG-6 reproduced the anti-inflammatory effects of MSCs both in vivo and in vitro and blockage of TSG-6 synthesis by MSCs removed any observed anti-inflammatory effects.[39]

MSCs might also have a therapeutic benefit in sepsis through reprogramming of host macrophages. In a series of well designed in-vivo experiments, Németh and colleagues[40] reported a therapeutic pathway in which MSCs exposed to TNFα or LPS increase production of prostaglandin E2 (PGE2). This pathway drives resident macrophages towards the M2 anti-inflammatory phenotype, thereby increasing their production of the anti-inflammatory cytokine interleukin 10 and causing decreased inflammation and inflammatory infiltration into tissue. Production of PGE2 by MSCs with induction of an anti-inflammatory phenotype in host immune cells has also been reported in vitro.[57] A summary of how the TSG-6 and PGE2 pathways contribute to our understanding of the anti-inflammatory potential of MSCs was published by Prockop in 2013.[58]

Ex-vivo human lung models

Although the anti-inflammatory effects of MSCs have not been tested in clinical trials, these effects have been studied in an ex-vivo isolated perfused human lung model. Clinical-grade MSCs were given, either into the lung perfusate or by direct instillation into the right middle lobe, 1 h after injury with intrabronchial *Escherichia coli*.[41] MSCs decreased neutrophil influx and almost completely restored normal lung histology. Similar effects were reported when the model was extended to 10 h after injury and a higher bacterial load was used. Intrabronchial FGF7 replicated the reduction in neutrophil influx seen with MSCs, suggesting a potential role of FGF7 as a paracrine factor, possibly by reduction of endothelial and epithelial permeability.

Regulation of endothelial cell permeability

Vascular endothelial injury is a defining characteristic of both ARDS[59] and sepsis.[53] MSC therapy might help preserve endothelial barrier function in both syndromes (figure).

MSCs and conditioned media from a co-culture of endothelial cells and MSCs have been reported to decrease endothelial paracellular permeability and protect against inflammatory disruption of barrier function in vitro by mobilisation of adherens junction proteins to cell membranes[42] and limitation of binding of inflammatory cells to the endothelium.[43]

In vivo, by use of a rat model of controlled haemorrhage, MSCs were seen to stabilise endothelial cells in haemorrhagic shock, partly by preservation of adherens junction and tight junction proteins.[43] MSCs were also shown to decrease vascular permeability in a mouse model of caecal ligation and puncture.[40] Finally, MSCs had beneficial effects on endothelial cells in studies using ex-vivo perfused human lungs.[44] MSCs and MSC-conditioned media, instilled intrabronchially 1 h after direct injury with *E coli* endotoxin, restored lung endothelial cell permeability to control levels.[44]

Regulation of epithelial cell permeability

The alveolar epithelial lining is composed of type I and type II alveolar cells. Alveolar epithelial cell injury contributes to several injury pathways in the development of ARDS, including loss of alveolar–capillary barrier integrity, dysregulated vectorial transport of alveolar fluid, and disordered surfactant production.[59] MSCs might have a role in the preservation of epithelial cell function in ARDS.

In vitro, co-culture of MSCs with human alveolar type II cells exposed to cytomix (a mixture of the proinflammatory cytokines interleukin 1, TNFα, and interferon γ) restored epithelial cell protein permeability to pre-injury concentrations without the need for direct contact between cells, which suggests a therapeutic effect via a paracrine mechanism.[45] Angiopoietin-1 (ANG-1), an angiogenic factor known to stabilise endothelial cells during injury,[60] seemed to be at least partly responsible for this improvement.

Similar findings were described in an in-vitro study of rat alveolar epithelial cells injured with cytomix and hypoxia.[46] Exposure of the injured cells to MSC-conditioned media restored normal epithelial barrier function. Concentrations of IL-1ra and PGE2 were noted to be statistically significantly increased in MSC-conditioned media after exposure to hypoxia and cytomix, suggesting their potential role as paracrine factors.

Increased alveolar fluid clearance

Removal of alveolar oedema fluid via vectorial transport across alveolar epithelial cells is crucial to recovery from acute lung injury.[61] A growing body of scientific literature suggests that MSCs improve alveolar fluid clearance, partly through an FGF7-mediated mechanism.

In an in-vitro model of epithelial cell injury using rat alveolar epithelial cells exposed to cytomix and hypoxia, incubation of injured epithelial cells with MSC-conditioned media preserved epithelial sodium transport and prevented a decrease in apical expression of αENaC subunits (one of the three subunits that form the epithelial sodium channel).[46] These benefits did not occur in FGF7-depleted MSC-conditioned media. Similar findings were reported in an in-vitro model with human alveolar type II cells exposed to cytomix.[44] Incubation of injured epithelial cells with MSCs preserved net fluid transport and partly restored apical membrane expression of αENaC subunits. Blockage of MSC FGF7 expression prevented this therapeutic effect, again suggesting that FGF7 is a probable epithelial-protective paracrine factor.

The ability of MSCs to restore alveolar fluid clearance has also been noted in ex-vivo perfused human lungs. Intrabronchial administration of both MSCs and MSC-conditioned media to lungs injured with *E coli* endotoxin has been shown to reduce lung water and normalise alveolar fluid clearance.[44] FGF7-depleted media had a negligable effect on alveolar fluid clearance, whereas the addition of recombinant FGF7 to the media restored its therapeutic benefit. Similar improvements in alveolar fluid clearance with MSCs were noted when ex-vivo lungs were directly injured with live bacteria.[41] Finally, in a 2014 study[47] with perfused lungs that were rejected for transplant, intravenous administration of MSCs normalised alveolar fluid clearance. Pretreatment of the perfused lung with an FGF7-blocking antibody statistically significantly reduced this effect.

Antimicrobial effects

Despite their immunosuppressive properties, MSCs have been reported to have several antimicrobial effects. Since infection is the most common cause of ARDS,[54] these antimicrobial effects raise important therapeutic possibilities for ARDS and sepsis.

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In murine infection models, MSCs reduce bacterial levels in the alveoli, blood, and spleen.[37,38,48–51] This antibacterial effect is partly mediated by improved phagocytic activity of host immune cells such as macrophages,[38,41] monocytes,[48] neutrophils,[49] and ITGAM-positive cells (monocytes, macrophages, and neutrophils).[38]

Studies using ex-vivo human lungs have reported similar findings. MSCs reduced alveolar bacterial counts and improved alveolar macrophage phagocytosis after direct bacterial injury.[41] This effect seems to be partly mediated by FGF7, because the use of an FGF7-neutralising antibody nullified the antimicrobial effects of MSCs both in vitro and ex vivo.[41] Alveolar fluid from lungs treated with MSCs was noted to have increased antimicrobial activity against *E coli* in vitro, suggesting the presence of a secreted antimicrobial factor.

In addition to FGF7, several other antimicrobial paracrine factors have been identified. In vitro, mouse MSCs have been reported to increase the production of the antimicrobial peptide lipocalin-2[51] and human MSCs produce LL-37[50] in response to infectious and inflammatory stimuli. Use of a blocking antibody for both of these peptides nullified the antimicrobial effects of MSCs in vivo.[50,51]

Anti-apoptotic effects

Apoptosis of both immune and structural cells is an important component of ARDS and sepsis pathogenesis.[53,62] A potential effect of MSC therapy is the ability to restrict the apoptosis of host cells. In vitro, both MSCs and MSC supernatant have been reported to have notable anti-apoptotic effects on resting and activated neutrophils.[52] This effect does not require direct contact between cells and seems to be mediated partly by MSC production of the anti-apoptotic cytokine interleukin 6 (figure).[52] MSC production of FGF7 has also been postulated to inhibit apoptosis of monocytes, leading to increased bacterial killing.[41] Future research will hopefully illuminate the extent and significance of this pathway.

Alternative pathways

Although the paracrine pathways described undoubtedly have a major role in mediation of the interactions between MSCs and host tissue, other potential pathways have been identified. Research investigating these pathways will probably contribute substantially to a more nuanced understanding of the mechanisms underlying MSC therapy.

Clear evidence exists that marrow-derived MSCs have a crucial role in regulation of the haemopoietic micro-environment in bone marrow[63] and can help to direct the creation of vascular networks in host tissue.[64] However, it is unclear to what extent the beneficial effects of MSC therapy for non-skeletal pathology might be secondary to this ability to interact with nascent capillary networks.[64]

Evidence also suggests that MSCs might modulate endogenous repair mechanisms and affect the activity of host progenitor cells.[65] MSCs express high levels of genes essential to the regulation of haemopoietic stem cells,[66] stimulate proliferation of endogenous cardiac progenitor cells during experimental myocardial infarction,[67] and possibly increase the number of lung progenitor cells in response to injury.[65]

Finally, MSCs seem able to affect tissue repair through the delivery of extracellular vesicles[68,69] and direct mito-chondrial transfer.[70] Although a detailed exploration of this scientific literature is beyond the scope of this review, table 5 shows a brief overview of several representative studies.

Challenges and future directions

In the past two decades, substantial progress has been made in the understanding of how MSCs interact with

	Summary
Extracellular vesicles	
Bruno et al[68,71]	Microvesicles derived from human MSCs had protective effects in both in-vitro and in-vivo acute kidney injury models
Lee et al[72]	Intravenous administration of MSC-derived exosomes decreased the influx of inflammatory mediators and inhibited vascular remodelling and pulmonary hypertension in a murine model of hypoxia-induced pulmonary hypertension
Zhou et al[73]	Renal capsule injection of exosomes isolated from human umbilical cord blood MSCs attenuated blood and histological markers of acute kidney injury in an in-vivo rat model; exosomes also limited apoptosis and oxidative stress in vitro
Zhu et al[69]	Intratracheal and intravenous delivery of microvesicles isolated from human MSCs reduced inflammation and lung water in a murine lung injury model using *Escherichia coli* endotoxin; in-vitro microvesicles restored epithelial cell barrier function after inflammatory injury
Mitochondrial transfer	
Islam et al[70]	In a murine lung injury model using intratracheal LPS, MSCs attached to alveoli and formed nanotubes through which mitochondria-containing microvesicles were transferred to the alveolar epithelium; this transfer ameliorated lung injury
Ahmad et al[74]	In-vitro and in-vivo evidence of mitochondrial transfer between MSCs and injured epithelial cells via nanotubes which rescues epithelial cells from inflammation and improves host bioenergetics
Li et al[75]	Mitochondrial transfer between human MSCs derived from induced pluripotent stem cells and lung epithelial cells injured by cigarette smoke both in vitro and in an in-vivo rat model

MSC=mesenchymal stem (stromal) cell. LPS=lipopolysaccharide.

***Table 5:* Representative studies lending support to novel MSC therapeutic pathways**

host tissue. However, a review of the translational promise of MSC therapy needs to be tempered with a summary of ongoing challenges in MSC research and gaps in knowledge (panel).

Despite thousands of published articles on MSCs, the terminology used to describe the cells being studied varies substantially. MSCs are referred to as skeletal stem cells,[76] marrow stromal cells,[77] mesenchymal stem cells,[8] multipotent mesenchymal stromal cells,[10] and even medicinal signalling cells.[78] Scientists continue to disagree over the most appropriate definition of MSCs with many following the criteria set out by the International Society for Cellular Therapy,[10] and others advocating the more conservative definition of marrow-derived cells able to generate a heterotopic ossicle in vivo.[79] MSCs are probably not true stem cells because they seem to have their therapeutic effects through mechanisms unrelated to their progenitor function and have not been convincingly shown to regenerate non-skeletal tissue.[79,80]

Beyond clarification of the phenotypes of MSCs, substantial research efforts are needed to fully identify the effects of MSCs when given to an injured host. As noted, our understanding of the paracrine effects of MSCs, their ability to interact with injured host cells, and their effect on host angiogenesis and endogenous repair is incomplete. Although the behaviour of MSCs is undoubtedly affected by the local microenvironment,[79,81] this effect cannot be reliably quantified and predicted.[13] Murine MSCs, although used in many preclinical models, have unique tumorigenicity and culture requirements, which raises questions about their ability to truly replicate the behaviour of human-derived MSCs.[13] Researchers also probably do not fully appreciate the inherent differences between MSCs cultured from different donors[82] and are only beginning to appreciate how age might affect MSC function.[83] Finally, we remain unable to answer definitively basic mechanistic questions, such as how MSCs have a therapeutic effect on non-pulmonary tissue when given intravenously. MSCs become trapped in the lung after intravenous administration, yet have beneficial effects in traumatic brain injury and myocardial infarction (supporting the presence of secreted paracrine factors).[80] All of these gaps in knowledge underscore a pressing need to validate candidate mechanisms in reproducible in-vivo models and for improved characterisation of bioactive factors and their mode of action.[79]

Two studies[84,85] in sheep models of ARDS have lent support to the safety and potential efficacy of MSC therapy. A small randomised trial of adipose-derived MSCs in 12 patients with ARDS in China is the first to suggest that MSCs can be safely given to patients with ARDS.[86] In the USA, a phase 1/2 clinical trial of a single infusion of allogeneic bone marrow-derived human MSCs in early ARDS, sponsored by the National Heart Lung and Blood Institute (NCTO1775774 and NCT02097641), is underway, while a Canadian phase 1 trial of MSC therapy for patients with septic shock (Cellular Immunotherapy for Septic Shock) is in the planning phase.

As the critical care community begins to focus on the use of MSCs in clinical trials,[8] researchers have to deal with a number of questions regarding drug safety, reproducibility, and clinical trial design. An emphasis on the need to ensure that preparations of MSCs used in clinical trials are of a standardised and verifiable quality is at the centre of many thoughtful reviews on the subject.[13,64,79,80,87,88] This requirement is challenging because many variables, such as temperature and culture density, can all affect MSC phenotype.[88] Furthermore, MSCs can be cultured from multiple sites, including adipose tissue, bone marrow, and muscle. Researchers do not yet understand how these cells differ biologically nor are they able to reliably quantify how these MSCs differ in their interactions with an injured host. Attempts to generalise the safety profile and therapeutic effects of a unique cell preparation should therefore be interpreted with caution. To help address these issues, experienced centres are now issuing MSC preparations prepared with standardised protocols.[80]

Although MSC therapy has been used in early clinical trials without apparent safety issues,[15,86,89] care should be taken when monitoring short-term and long-term safety. For trials including patients with heterogeneous diseases such as ARDS and sepsis, thoughtful inclusion criteria and reliable endpoints should be considered to obtain clinically meaningful results.[87] Finally, many issues remain with regard to clinical trial design such as determination of the optimum mode and timing of MSC delivery, and identification of which patients with ARDS or sepsis are most likely to benefit from experimental therapy.[8]

Panel: Ongoing challenges in mesenchymal stem (stromal) cell (MSC) translational research

- Improvement of our mechanistic understanding of how MSCs interact with host tissue
- Description of the importance of non-paracrine pathways, such as mitochondrial transfer and interactions with intrinsic progenitor cells
- Validation of candidate mechanisms in reproducible in-vivo models
- Elucidation of the effect of local microenvironments on MSC function
- Quantification of how donor site (eg, adipose tissue *vs* bone marrow) and age affect MSC function
- Improvement of our understanding of how cryopreservation and thawing affect MSC function
- Clarification of the optimal dose and delivery route for MSCs in clinical trials
- Investigation of the efficacy and safety of cell-free therapy

Search strategy and selection criteria

Articles for this Review were identified by searches of Medline, Current Contents, PubMed, and references from relevant articles using the search terms "MSC", "mesenchymal stem cells", "mesenchymal stromal cells", "marrow stromal cells", "acute respiratory distress syndrome", "acute lung injury", and "sepsis". Experts in the field were asked for additional or unpublished research not identified in the original search. We including only articles published in English between January, 1968 and August, 2014.

As the list of identified bioactive factors and extracellular vesicles secreted by MSCs continues to expand, the isolation of these molecules and investigation of their clinical use separate from MSCs (cell-free therapy) will be of increasing interest.[90] As with MSC therapy, a push for expedited clinical trials will need to be balanced with a focus on basic and translational research to improve the understanding of the in-vitro and in-vivo behaviour of cell-free therapies. Attention will need to be given to the full identification and classification of the bioactive molecules secreted by MSCs, determination of how cell-free therapies differ in both safety and efficacy (conditioned media *vs* isolated bioactive factors *vs* exosomes), and tests of whether potential therapies should be given as single drugs or in combination. Finally, new safety concerns will need to be carefully investigated, including the ability of exosomes to act as delivery vehicles for viruses and cancer proteins.[91]

Conclusion

The clinical use of MSCs has been variably described as a therapy likely to change the practice of medicine[78] and one inappropriately cast as a panacea for all disorders without the necessary supporting in-vivo research.[79] The many preclinical models reviewed suggest that MSC therapy holds substantial therapeutic promise for ARDS and sepsis, especially with the scarcity of viable pharmacological treatments. However, encouraging preclinical findings do not guarantee efficacy in clinical trials. Experimental studies and ongoing randomised trials will have an important role in the clarification of the therapeutic potential of MSCs and furthering our understanding of how MSCs interact with host tissue.

Contributors

All authors contributed equally to this work. MAM and JW provided the plan and wrote the Review. LBW provided editorial review and modified the report.

Declaration of interests

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