11 Preclinical Evidence for the Role of Stem/Stromal Cells in Targeting ARDS

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11.1 Acute Respiratory Distress Syndrome

Acute respiratory distress syndrome (ARDS) is a high-mortality syndrome that develops following an infection or trauma, leading to a dysregulated inflammatory response in the lung that can cause tissue remodeling, pulmonary dysfunction, and death [1]. ARDS is characterized by acute respiratory failure caused by an increase of fluids in the alveolar space. The breakdown of the immune response increases the permeability of the epithelial–endothelial barrier. This results in an increased filtration of protein-rich fluid from the vascular system into the alveolar spaces, with a subsequent lung edema and a decrease in the ability of gas exchange [2].

ARDS develops as a localized lung response that could have multiple origins including pneumonia, systemic sepsis, major surgery, or multiple trauma. Patients with ARDS have an acute onset of symptoms like severe chest pain and a decrease in their pulmonary function associated to pulmonary infiltrates in the X-ray, indicative of pulmonary edema. These symptoms usually appear after the first week of the injury [3].

ARDS accounts for more than 10% of intensive care unit (ICU) admissions worldwide and has a mortality rate of about 40% [4]. ARDS results in a diminished quality of life and lung function; and survivors often have long-term neuromuscular, cognitive, and psychological symptoms. Additionally, long hospitalization, ICU, and increased use of health care services after hospital discharge have enormous socioeconomical cost [5, 6].

Despite several decades of research, there is no disease-modifying therapy for ARDS. Because the mechanisms driving lung injury are complex and diverse, pharmacological treatments often fail, suggesting that targeting a single mediator or pathway is not enough to achieve therapeutic effects. As an alternative, after their use in several preclinical models of ARDS, cell-based therapies

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with mesenchymal stem/stromal cells (MSCs) are promising, as they can target multiple cellular and extracellular impairments associated with ARDS simultaneously [7].

11.2 Pathology of ARDS

There are multiple initiation agents for ARDS, from microorganism invasion (bacteria or viral) to mechanical stressors, that result from mechanical ventilation [8]. Beyond the initial trigger, aging, like in any other lung diseases, there is an impaired capacity of the lung to recover. In several animal models, when we compared aged with young, there was an increase in the morbidity and mortality as a consequence of an altered inflammatory response [9–11].

Immune activation results in the release of proinflammatory cytokines and chemokines with a primary influx of neutrophils into the alveolar space, leading to the release of metalloproteinases (MMP), myeloperoxidases (MPO), collagenase, and the generation of reactive oxygen species (ROS) [12]. This activates and attracts macrophages and lymphocytes to the site of injury with a sequent release of inflammatory cytokines including IL-6, IL-8, IL-1β, and TNFα [13].

In physiological situations, anti-inflammatory mediators act to limit the inflammatory cascade and control the tissue damage. ROS serves multiple functions such as killing phagocytosed microorganism, or the removal of cell debris and signaling. However, high and persistent levels of ROS, MMPs, and MPOs cause tissue necrosis, injury, and destruction [14]. The controlling feedback mechanism seems to be impaired during the onset of sepsis, which leads to a persistent inflammatory response after the resolution of the initial insult [15].

Along with the production of proinflammatory cytokines, other factors are secreted such as endothelin-1 (EDN1), angiotensin-2 (AGT-II), and phospholipase A2 (PLA2), which increase vascular permeability [16, 17]. The disruption of epithelial–endothelial barriers leads to a disruption of the alveolar clearance and production of surfactant [18]. Edema accumulates in the alveoli though the increase permeability of epithelial and endothelial barriers and the decrease in alveolar fluid clearance. Measurements of the protein content in BAL fluid provide an estimate of the alveolar changes and could be used as an indicator of the prognosis of the patient [19]. There is an initial phase of fluid accumulation followed by a proliferation phase, which is characterized by the increase of type II alveolar cells (AEC-II), fibroblast, myofibroblast, and matrix deposition. If the inflammation persists, then there is a disorganized repair that could lead to fibrosis [20, 21].

11.3 MSCs in the Treatment of ARDS

MSCs constitute a great option for the treatment of ARDS because of their ability to regulate the immune response, enhance the phagocytic clearance of bacteria and secrete factors that regulate the capillary–alveolar barrier. Additionally, MSCs appeared immune-privileged with low levels of type I HLA antigens in their surface, which allows them to escape from the patient's immune response [22, 23]. This represents an important advantage that allows the therapeutic use of allogenic MSC [24].

MSC could exert their effect through cell contact-dependent mechanisms and by the release of soluble factors. This chapter summarizes all the processes by which MSCs could be beneficial in the treatment of ARDS (Fig. 11.1) and the preclinical animal models of lung injury in which these have been tested.

11.4 Mechanism of Action of MSC

Tracking MSC engraftment has been possible using MSC expressing GFP or labeled with PKH allowing the localization of the cells by fluorescent imaging [25–27]. There is considerable variety in the number of cells homing into the lungs. We have demonstrated an early retention in the lung of MSCs in large animal models of acute lung injury independent of the way cells were delivered [28]. However, it is well accepted that

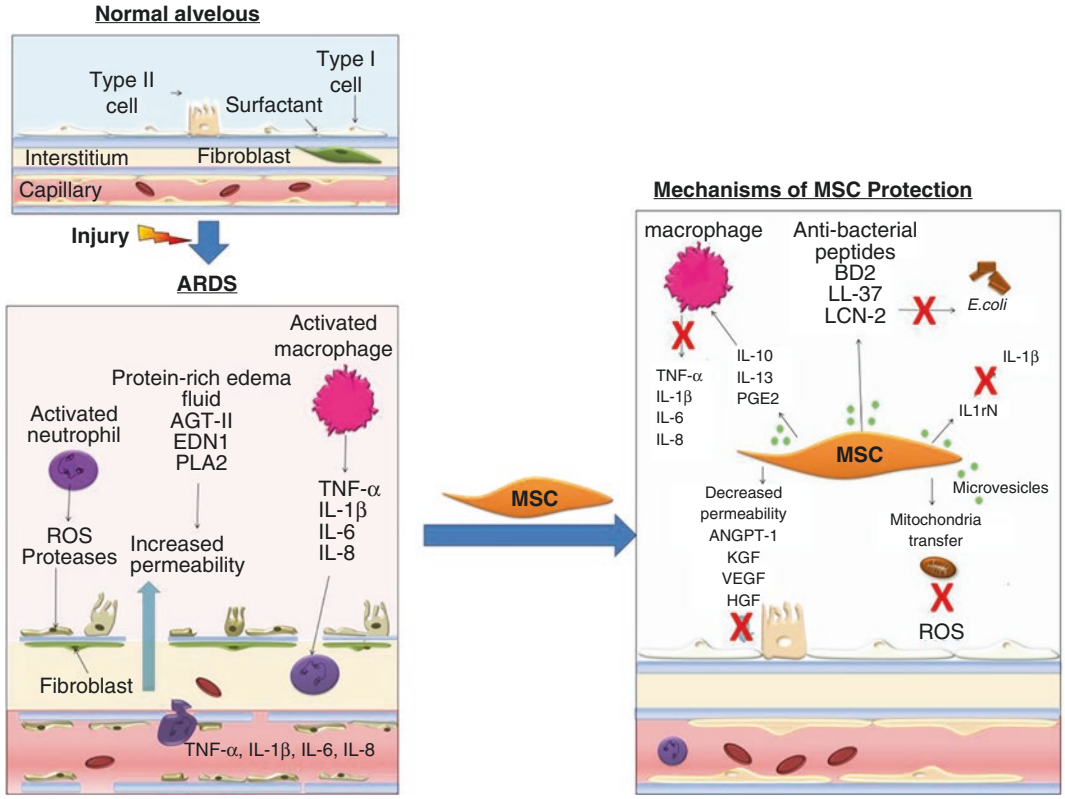

Fig. 11.1 Schematic representation of the mechanisms driving alveolar damage during ARDS and the different pathways targeted by soluble factors produced by MSCs and the consequences on lung repair

by using colocalization of surface markers, infused MSCs that localize in the lung are not differentiating into any other cell type, including alveolar or airway epithelial cells, fibroblasts, or endothelial cells. The actual knowledge suggest that most of the presence of MSCs observed in the LPS model of lung injury reflects a transient process [29] and that the protective effect seen with MSC therapy does not require MSC differentiation into any cell type [14]. Engineered MSC to overexpress important genes for the epithelial lineage like angiopoietin [30], ROR2 [31] or β-catenin [32] showed an enhanced differentiation capacity in vitro, but there was no engraftment and differentiation in in vivo models of LPS-induced acute lung injury.

Despite the initial interest in the multipotent properties of the MSCs, engraftment and differentiation in the lung [14, 33], their beneficial effect more likely derives from their capacity to be recruited by the sites of injury, interact with the host cells, and secrete soluble factors known as the secretome [34]. The MSC secretome is dynamic and could vary depending on the MSC source or the type of lung injury. It includes an extended array of bioactive molecules comprising cytokines, chemokines, growth factors, angiogenic factors, and microvesicles [2]. Table 11.1 summarizes the described molecules of the secretome and their main functions.

11.4.1 MSCs Reduce Endothelial and Epithelial Permeability

The integrity of the microvascular endothelium is essential to avoid the influx of protein-rich fluid from the circulation to the alveolar space. In addition to the generated edema, this permeabilization comes with inflammatory cytokines and

Table 11.1 Secretome of the MSCs and their therapeutic actions against ARDS

Molecule	Mechanism of action
Reduce endothelial–epithelial permeability	
Angiopoietin-1 (Ang-1) [35, 36]	Promotes endothelial survival, reduces permeability, and inhibits leukocyte interactions
Keratinocyte growth factor (KGF) [37–41]	Promotes epithelial proliferation, may be attribute for the promotion of AEC-II and the production of surfactant
Hepatocyte growth factor (HGF) [42–46]	Preserve integrity of the endothelium
Sphingosine-1-phosphate (S1P) [47, 48]	Enhances the resistance of the endothelial barrier and reduces the levels of TNFα
Vascular endothelial growth factor (VEGF) [45, 49–51]	Reduces lung permeability, protects endothelium from apoptosis, controls inflammation, and facilitates VE-cadherin recovery
Alveolar fluid clearance	
KGF [37–41]	Increases trafficking of sodium transport proteins to the cell surface, reduces aquaporin 5 expression, and increases epithelial repair
Fibroblast growth factor 7 (FGF7) [52]	Increases expression of sodium channels
Epithelial growth factor (EGF) [53, 54]	Stimulates the proliferation of epithelial cells, increases sodium channels and Na-K-ATPase function
TGF-β [55, 56]	Increases activity of the sodium channels
Immune response regulation	
IL-4 [57, 58]	Anti-inflammatory cytokine inhibiting type I responses
IL-10 [59, 60]	Anti-inflammatory cytokine inhibiting neutrophil recruitment and activation
IL-13 [61]	Anti-inflammatory cytokine
IL-1rN [62]	Competes with IL-1β receptor binding, inhibiting its effects
Prostaglandin E2 [63, 64]	Stimulates macrophages to produce IL-10
KGF [37, 41]	Induces the secretion of granulocyte–macrophage colony-stimulating factor that increases alveolar macrophage phagocytosis and inhibits macrophage apoptosis
TNF-stimulated gene protein 6 (KGF6) [65, 66]	Anti-inflammatory cytokine
Bacterial clearance	
β-Defensin 2 (BD2) [67]	Inhibits bacterial growth in vitro
LL-37 [68, 69]	Antibacterial, antifungal, and antiviral properties
Lipocalin-2 [70–72]	Regulates chemokines such as CXCL9 to reduce inflammation in front bacterial infections

cells which may further aggravate the ability of the endothelium to reduce edema [33]. Several MSC-secreted factors have the capacity of regulate the alveolar microvasculature reducing its permeability.

Angiopoietin-1 (Ang-1) is a ligand for endothelial Tie2 receptor activating the NF-κB pathway to prevent the formation, on AEC-II, of actin stress fibers and preserve the localization of claudin-18 [73]. Ang-1 has been shown to promote endothelial survival, reduce endothelial permeability, and inhibit leukocyte interactions by modifying cell adhesion molecules and cell junctions [36, 74, 75]. Engineered MSCs overexpressing Ang-1 further reduce protein content, albumin, and immune cells in BAL [35, 76, 77]. MSC culture in proinflammatory conditions enhances their capacity to produce Ang-1. Also, when Ang-1 was blocked with siRNA, MSCs no longer prevented epithelial permeabilization [73].

Keratinocyte growth factor (KGF), also known as fibroblast growth factor 7 (FGF7), is a critical factor for epithelial repair and stimulating epithelial cell proliferation [41]. Animal models of ALI, such as by administration of α-naphthylthiourea [37, 52] *P. aeruginosa* [78] or ventilator-induced lung injury [39], have shown the ability of KGF to reduce alveolar edema. Engineered MSCs overexpressing KGF improve microvascular permeability, reduce proinflammatory cytokines (IL-1β

and TNFα), and increase the anti-inflammatory response (IL-10). The underlying mechanism is not completely understood but may be attributed to the promotion of AEC-II cells and the production of surfactant [41].

Hepatocyte growth factor (HGF) was found to preserve integrity of pulmonary endothelium though the inhibition of Rho GTPase, prevent the actin stress fiber formation, and preserve the gaps between endothelial cells [39, 42]. Sphingosine-1-phosphate (S1P) enhances the resistance of the endothelial barrier. This mechanism seems to be dependent of the capacity to inhibit leukocyte permeability as well as reduce levels of TNFα [47, 48]. Vascular endothelial growth factor (VEGF) has been described to reduce lung permeability, protect endothelium from apoptosis, control inflammation, and facilitate VE-cadherin recovery. Knockdown of VEGF in MSCs confirms these activities as these cells present reduced therapeutic activity on ALI models [51].

11.4.2 Alveolar Fluid Clearance, AFC

Alveolar fluid clearance (AFC) is the capacity of the lung epithelium to remove alveolar fluid during pulmonary edema. This process is mediated through sodium channels, aquaporin, and sodium-potassium adenosine triphosphatase Na-K-ATPase [43]. Many conditions such as high-volume mechanical ventilation, live bacteria, or proinflammatory cytokines can reduce AFC [79, 80]. The compromised capacity of AFC is used as prognostic value to determine morbidity and mortality [80]. Both AEC-I and AEC-II are involved in AFC during pulmonary edema. AEC-I has the highest permeability to water, potentially through aquaporin, which supports its role in ion transport [81–83]. Na-K-ATPase is expressed in both AEC-I and AEC-II and has a critical role in alveolar fluid reabsorption, where the sodium transport is followed by outflux of water in an isosmolar manner [84, 85].

Studies with intra-bronchia administration of LPS in ex-vivo perfused lungs revealed a marked decrease in AFC, and mechanisms dependent on the blood presence suggest that immune cell are required for the injurious effect of the LPS. MSCs, or their conditioned media (CM), normalized AFC in a KGF-dependent manner as siRNA for KGF reduced the therapeutic effect of the MSCs [86]. KGF increases fluid transported across the alveolar epithelium through increased trafficking of sodium transport proteins to the cell surface [87, 88]. KGF also has important functions reducing the expression of aquaporin 5 and avoiding the transdifferentiation of AEC-II toward AEC-I [38]. Another group described that KGF effects on AFC are mediated through an increased rate of epithelial repair, cell adherence, and migration [40].

Epithelial growth factor (EGF) stimulates the proliferation of epithelial cells, increases sodium channels and Na-K-APase function in in vitro alveolar epithelial cultures [54]. An in vivo rat model with aerosolized EGF showed an increase in active sodium transport, Na-K-ATPase activity, and lung fluid clearance [53]. Rat models with instilled TGF-β increase alveolar fluid clearance in a time-dependent and dose-dependent manner. This increase of alveolar clearance is driven by an increased activity of the sodium channels [55].

11.4.3 Immune Response Regulation

MSCs constitutively do not exert their immunomodulatory properties but instead have to be "primed" by inflammatory mediators [89]. In the context of ARDS, the acute inflammation drives this "priming" of the MSCs, activating their immunomodulatory properties [24]. MSCs were found to protect tissue damage from extraordinary inflammation by downregulate the expression of proinflammatory cytokines as IL-6, IL-8 IL-1β, IFNɣ, and TNFα and through the production of anti-inflammatory cytokines such a IL-4, IL-10, or IL-13 [71, 79, 90, 91]. Additionally, MSCs produce IL-1rN which is a cytokine that competes with IL-1β receptor binding, thus inhibiting its effects [62].

Several in vitro and in vivo studies have demonstrated that MSCs have several effects over the innate immune system. They can influence the

maturation of the dendritic cells (DC) [92], increase the phagocytic capacity of monocytes [93–95], neutrophils [96–98], and modify macrophage toward immunomodulatory M2 phenotype [99, 100]. MSCs secrete prostaglandin E2 that then stimulates macrophages and monocytes to produce the anti-inflammatory cytokine IL-10 [101]. IL-10 has been reported to inhibit the rolling, adhesion, and transepithelial migration of neutrophils, thus resulting critical downstream in the MSC therapeutic effects [13, 102]. MSCs produce KGF that induces the secretion of granulocyte–macrophage colony-stimulating factor that increases alveolar macrophage phagocytosis [103]. KGF also inhibits macrophage apoptosis through a downregulation of the β-catenin pathway that increases Bcl-2 and decreases BAX and caspase-3 [104].

MSCs also can modulate the adaptive immune response. MSCs suppress B cell proliferation and terminal differentiation [105], suppress T cell proliferation [102, 106, 107], induce a switch from Th1 proinflammatory response to Th2 response, and finally increase the number of T regulatory cells [108]. MSCs secrete idoleamine 2,3-dioxygenase (IDO) upon stimulation with IFNɣ. IDO activity results in tryptophan depletion and kynurenine production that inhibits T cell proliferation [109]. TNF-stimulated gene protein 6 (KGF6) is another contributor to the immunomodulatory effects of the MSCs. In lung injury models, MSCs upregulate KGF6 that binds IL-8, blocking its function [66]. Blockage of KGF6 by siRNA completely reverses MSCs' anti-inflammatory properties [65].

It is important to note that while MSCs secrete an extensive range of anti-inflammatory cytokines, they also have the capacity to produce several proinflammatory cytokines such as IL-6 and IL-8. The production of these cytokines has been associated with poor outcomes in ARDS patients [110, 111]; however, there is new evidence that suggests a role of these cytokines in the MSC therapeutic effect. IL-6 is usually implicated in proinflammatory responses, but apparently, it could have promiscuous functions [112–114]. It is not clear how IL-6 induces therapeutic effects, but its inhibition in lung injury models diminished the MSC therapeutic effect [115]. The role of IL-8 is not clear but there is evidence suggesting that IL-8 promotes the production of VEGF that promotes angiogenic effects [50].

11.4.4 Enhancement of Bacterial Clearance

MSCs attenuate bacterial sepsis directly by antimicrobial peptides secretion and by enhancement of macrophage phagocytosis [116]. MSCs stimulated by the presence of bacteria released β-defensin 2 (BD2) [67], LL-37 [68, 69] and lipocalin-2 [71]. BD2 is upregulated in the presence of bacteria through TLR2 and TLR4 pathways, inhibiting bacterial growth in vitro [67]. LL-37 is a cathelicidin peptide with antibacterial, antifungal, and antiviral properties [68, 117]. Lipocalin-2 is known to act on mucosal cells during pulmonary infection to regulate chemokines such as CXCL9 to reduce inflammation in front bacterial infections [70, 72, 117].

CM from stimulated MSC was found to contain high levels of antimicrobial peptides and to inhibit bacterial growth. However, when treated with inhibitors of these peptides, the effect was abolished. Animal models of lung injury show a reduced bacterial growth in the lung homogenates on the animals treated with MSCs. This effect was decreased if a neutralizing antibody against LL-37 was administered with the MSCs [92]. Similar observations were done blocking the TLR2 and TLR4 and thus the production of BD2 [67].

11.4.5 Transfer and Rescue of Mitochondrial Function in Target Cells

MSCs could contribute to rescue mitochondrial function in epithelial cells containing nonfunctional mitochondria [118, 119]. In vitro imaging reveals the formation of connexin-43-gap junctions between MSC and alveolar epithelial cells, allowing the transport of mitochondria to the LPS-injured epithelium. This resulted in increased ATP levels that rescued the surfactant

secretion by AEC-II leading to reduce alveolar permeability and reduced mortality. An endotoxin lung injury model treated with instilled MSCs confirmed this mitochondrial transfer to the injured epithelium, leading to the restoration of its function. This rescue mechanism was abrogated when MSCs with dysfunctional mitochondria were instilled, supporting its role in restoring the lung epithelium [25]. As described in more detail in Chap. 4, another group found that the mitochondria were transferred through nanotubes, which was upregulated by the Rho-GTPase Miro1 [120].

Mitochondrial transfer to macrophages has also been described in vitro through the formation of tunneling nanotubes. Mitochondrial transfer enhanced macrophage oxidative phosphorylation and phagocytosis. The blockage of the tunneling nanotubes formation completely abolished the MSC effect on macrophages [121, 122].

11.5 Preclinical Models of MSCs in ARDS

11.5.1 Animal Models of ARDS Treated with MSCs

The therapeutic efficacy of MSCs in the treatment of lung injury has been demonstrated in numerous animal models. Most of the studies have been performed in mouse or rat models and large animal models using swine [145, 146] or sheep [147, 148]. Acute lung injury is mainly induced through infection with endotoxin or live bacteria, and via cecal ligation and puncture driving sepsis. Rat models are preferred for the study of ventilator induced lung injury. There is lot of variability in dose, timing, route of administration, and source of MSCs, but all the studies found an improvement of the injury. Table 11.2 shows the main findings in animal models of ARDS using MSCs.

Animal models have shown the potential of using MSCs as a treatment therapy for ARDS. A recent study compared the therapeutic potential and the distribution of the MSCs with different administration routes in a large animal model of acute lung injury. Endobronchial and intravenous administration of MSCs have similar rates of lung retention at 5 h post administration and recovery of arterial oxygenation in equal extent [28].

Most of the studies performed showed histological improvements upon MSC treatment with reduction of the inflammation, edema, and lung injury with reduced collagen deposition [29, 65, 69, 71, 94, 108, 123–138]. At the endothelial–epithelial level, the alveolar barrier is better maintained in the animals treated with MSCs, with lower levels of protein in the BAL [65, 123, 129, 135–137, 139, 140]. In vivo, MSCs display immunomodulatory properties modifying the lung immune response. At molecular levels, MSC are able to shift the cytokine profile from the pro-inflammatory status in ARDS toward a more anti-inflammatory one [124, 128, 131, 133, 135, 136, 138, 140, 141]. At the cellular level, MSCs promote a T regulatory lymphocyte response [108], increasing phagocytic activity of macrophages [69, 101, 127, 129] and reducing the neutrophil numbers [8, 125, 127, 131, 136]. All these modifications of the immune response control the inflammation and preserve the lung tissue integrity. Also, MSC perform antimicrobial properties promoting phagocytosis and bacterial killing by the secretion of LL37 peptide [69] or lipocalin [71, 94]. In addition, MSC have showed antioxidant effects in vivo, preserving plasma levels of cysteine and glutathione redox state after endotoxin administration [126, 149].

11.5.1.1 Development of Genetically Modified MSCs

MSCs' capacity to migrate to the sites of inflammation makes them an attractive factor for gene-based therapy [150]. Overexpression of specific genes in MSCs can be used to enhance their therapeutic effects [25]. Most of the approaches are directed to increase their regenerative potential or their immunomodulatory capacity.

Administered MSCs overexpressing KGF improve pulmonary microvascular permeability, reducing the lung injury in the model of LPS-induced lung injury [41]. VEGF-overexpressing MSCs protect the endothelium from apoptosis, reducing the permeability and edema [51]. The

Table 11.2 Main therapeutic findings in animal models of ARDS

Lung findings	BAL findings	Plasma findings
Physiological changes		
Prevention of lung inflammation, injury, and edema [29, 65, 69, 71, 94, 108, 123–138] Increased adhesion molecule-1 and vascular cell adhesion molecule-1 [134, 139] Increased antioxidant and reduced glutathione [126] Reduced elastase [129, 135–137, 139] Reduced collagen [129] Increased KGF [139] Mitochondrial transfer [25]	Reduced protein content [65, 123, 140]	Improved oxygenation [135, 137]
Anti-inflammatory		
Cytokines: IL-1β, TNFα, and IL-6 [124, 128, 131, 133, 135, 136, 138, 140, 141] Increased anti-inflammatory cytokines: IL-10 and IL-1RN [130, 131, 133, 135, 138, 140] Reduced neutrophils [8, 125, 127, 131, 136] Reprogramming of macrophages to M2 [69, 101, 127, 129] Increased Tregs [108]	Cytokines: TNFα, MIP2, IL-1β, and IL-6 [65, 123, 124, 130, 137, 139, 140, 142] Increased IL-10 [123, 142] Reduced neutrophils [65, 124, 137] Reduced cell count [130, 137, 139, 140, 142]	Decreased expression of IL-1β, TNFα, and MIP2 [8, 29, 123, 124, 132, 141, 143] Increased expression of IL-10 [8, 143] Increased phagocytic activity of monocytes [141]
Antimicrobial		
Promotion of phagocytosis and bacterial killing [68, 69, 124, 141] Reduced viral load [13, 144] Increased LL37 in rat lungs [69]	Reduced bacterial growth [68] Increased LL37 [68] Enhanced bacterial clearance by production of lipocalin-2 [71, 94]	Reduced bacterial counts [141] Increased LL37 [69]

expression of HGF makes MSC to protect adherent junctions, VE-cadherin of the epithelium and reduce apoptosis, preserving lung architecture [46]. β-Catenin-overexpressing MSC improves alveolar permeability, promoting the differentiation of lung precursors into AEC-II [32]. Engineered MSCs targeting the immune system have been modified to express anti-inflammatory molecules or to alter the expression of chemokines. MSCs overexpressing prostaglandin receptor [63], IL-10 [151], or IL-1rl1 [152] induce a strong shift in the cytokine profile toward an anti-inflammatory response, reducing lung inflammation and edema. Finally, MSCs with altered expression of chemokine receptors show a reduced accumulation in the lung of inflammatory cells and mediators [153].

All the previous studies described the effects of the MSC in acute lung injury when administered at the initiation of the injury, but administration of MSCs once the injury is already performed did not show significant therapeutic effects [154]. MSCs overexpressing the microRNAs let-7d (antifibrotic) or miR-154 (profibrotic) were administered 7 days after bleomycin instillation. Mice treated with let-7d expressing MSCs were found to recover quicker from the initial weight loss, while those untreated or treated with miR-154 had the lowest survival rate, although no fibrotic differences were found in the lung tissue. The effect was more immunomodulatory, altering the pattern of cytokines and the leukocyte infiltration [155].

11.5.1.2 Pretreatment of the MSCs to Enhance Their Potential

MSCs' immunomodulatory effects vary depending on the immune microenvironment [156, 157]. The therapeutic effect achieved varies greatly between different studies and one possible explanation could be the resting status of the MSC. The immunosuppressive function of the MSC is enhanced by the presence of pro-inflammatory cytokines such as IFNɣ, TNFα, IL-1α, or IL-1β

[158], while the presence of anti-inflammatory cytokines like IL-10 abrogates their suppressive effect and even induces the production of pro-inflammatory cytokines [159–161].

An early study assessing the role of the microenvironment in the treatment of acute lung injury with MSCs showed the importance of the expression of TLR4 on the MSCs. TLR4 expression of MSCs is essential for the release of prostaglandin E2 upon activation by LPS or TNFα. MSCs lacking the genes for TLR4 or their downstream mediators are unable to produce prostaglandin E2 and activate macrophages to produce IL-10 in a mouse model of acute lung injury [101].

Rojas et al. showed that MSC treatment with serum from ARDS patients containing pro-inflammatory cytokines increases their immunomodulatory function with higher production of IL-10 and IL-1β receptor agonist. Pretreated MSCs have an enhanced protective capacity, reducing lung injury, edema, and accumulation of pro-inflammatory cells and cytokines in a mouse model of acute lung injury [130].

The time point at which MSCs are administered seems to be crucial. Only MSCs injected at the time of inducing the lung injury have shown to have therapeutic effect, even though they could lose all the antifibrotic properties when administered long after the injury [154]. One of the possible mechanisms responsible for the shift in their function is the microenvironment of the injured lung. In a mouse model of irradiated lungs, TGF-β expression was incremented in the damaged lungs, and in vitro the cytokines released by the lung injured cells inhibited the differentiation of the MSC into epithelial cells [162]. A recent study of acute lung injury by acid instillation revealed that treatment with MSC worsens the acid effects, driving a fibrotic process. This process seemed to be mediated by the presence of IL-6 and fibronectin in the microenvironment, which could be driving a senescence-associated phenotype on the MSCs. The fibrotic effect was reversed when the MSC were engineered to overexpress IL-10 or HGF neutralizing both in vivo in mouse models of HCl and ventilator-induced lung injury [163].

Another factor that could influence the therapeutic effect achieved is the aging status of the MSCs. MSCs isolated from aged individuals have reduced expression of cytokine and chemokine receptors that impair their migration and activation, failing to reduce inflammation in a mouse model of acute lung injury [164].

11.5.1.3 Alternative Sources of MSCs Than Bone Marrow

Traditionally, MSCs obtained from bone marrow have been used in most of the preclinical and translational studies. However, there is a growing number of studies using adipose and umbilical cord blood (UC-MSC) as potentially more plentiful sources. MSCs from different sources exhibit different receptors and immunomodulatory properties, which may cause differential therapeutic effect on ARDS, but overall, animal models have shown beneficial outcomes [165–171].

UC-MSCs have shown higher proliferative rates and lower expression of senescence markers than BM-MSCs which could reflect a more multipotent capacity [165]. UC-MSCs have great immunomodulatory capacity in mouse models of acute lung injury, inducing a shift toward a regulatory immune response with increasing levels of IL-10 and phagocytic macrophages [60, 64]. At the regenerative level, UC-MSCs attenuate lung injury, preserving vascular permeability and protecting from apoptosis [56, 172, 173].

Adipose MSCs have the advantage of their availability and easy isolation. Adipose MSCs have shown immunomodulatory properties in animal models of acute lung injury reducing inflammation, leukocyte infiltrate and modifying the cytokine profile toward an anti-inflammatory response [59, 174–176]. Recently, other studies have appeared using stem cells from pulp and periodontal ligament or menstrual stem cells, showing improvement of alveolar epithelial permeability and reducing pro-inflammatory cells and cytokines in LPS-induced ARDS models [177, 178].

11.5.1.4 Use of Soluble Factors Generated by MSCs

Microvesicles (MV) are small circular membrane fragments that are shed from the cell surface or released from the endosomal membrane and play an important role in cell communication. This communication system has emerged early during

evolution and serves as template in the further development of intercellular interaction mechanisms. MV can transfer specific genes, miRNAs, or small organelles, including mitochondria, from the MSC to the injured target cell through the connexin-43 gap junction channels.

MSC have demonstrated to have therapeutic effects in in vivo animal models but little is known about their long-term side effects, including the possibility of becoming tumorigenic. Given that the MSCs' therapeutic effect depends on the release of soluble factors, the in vivo use of MV represents an alternative and safer approach. Analysis of the RNA of microvesicles derived from MSCs revealed mRNAs associated with transcription, proliferation, immune cell regulation, and microRNiAs as well.

Intra-tracheal instillation of MSC-derived MV reduces edema and alveolar protein levels in mouse models of acute lung injury [179]. MV also show anti-inflammatory properties reducing neutrophils and inflammatory macrophages. A partial therapeutic effect of MSC MV depends on KGF, as KGF siRNA pretreatment of MSCs partially eliminated their therapeutic benefits [180]. Further studies show that the MV-dependent activity is mediated by CD44 receptors, promoting internalization of MV into monocytes, resulting in a decreased expression of inflammatory cytokines [181].

11.5.2 Ex-Vivo Lung Perfusion (EVLP) Models

Ex-vivo lung perfusion (EVLP) offers a unique opportunity for in situ testing of the effects of MSCs. EVLP was originally developed as a treatment to increase the number of lungs available for transplantation. Nowadays it is not only a way to improve unacceptable lungs for transplantation, but it also represents an excellent research tool as a preclinical model for in situ testing [182]. Several groups have developed models of acute lung injury [86, 103, 183] in which the effect of MSCs was studied [86, 103, 184, 185].

A study conducted on pigs looked at the optimal route and dose for the MSCs. Intravascular delivery of MSC showed better outcomes that intratracheal administration and the optimal dose was of 5×10^6 MSC per kilogram of animal [184]. Early studies using MSCs in human lung grafts were focused on testing their capacity to restore the AFC in lungs that were unsuitable for transplantation. Intravenous administration of MSC restored AFC in injured lungs in a mechanism dependent on KGF [185].

A couple of groups have developed models of ARDS to study the disease in human lung grafts. Treatment with MSC reduces edema, improves AFC, and restores epithelial barrier permeability in ex vivo perfuse human lungs injured with *E. coli* endotoxin. The beneficial effect on endotoxin injured lung was almost abolished when the MSCs or their conditioned media (CM) was treated with KGF siRNA [86]. The treatment with MSC in a model of pneumonia using live bacteria restored AFC, reduced inflammation, and increased bacterial killing through increased macrophage phagocytosis. KGF was shown as one of the main factors protecting monocytes from apoptosis and increasing bacterial clearance [103]. A recent study used MSC in combination with extracorporeal membrane oxygenation (ECMO) after inoculation of *E. coli* endotoxin in a sheep model. The combination of the MSC and the ECMO treatment showed better histopathology changes with less inflammation [186].

The use of MSCs microvesicles has a positive result as well in the treatment of injured lung grafts. Human MSCs derived microvesicles have been used to recover lungs rejected for lung transplantation by increasing AFC and improving airway and hemodynamic parameters [187]. In another study, treatment of ex-vivo lung perfusion model of bacterial pneumonia with MSC microvesicles increased AFC, reducing protein permeability and bacterial load [188].

In summary, since the description of the protective effect of the administration of MSCs to mice with induced ARDS [29, 123], several research groups have confirmed this observation on small and large animal models and more recently in the EVLP in which ARDS is induced in human lungs. The proposed mechanisms by which MSCs can induce protection are multiple,

some described in the present chapter. However, there is consensus that engraftment in the lung and differentiation into lung cells does not occurs. More recently, data generated from approved clinical trials, conducted by several academic institutions, demonstrated that the use of MSCs is safe and, in some cases, with demonstrated protection in patients with ARDS. There is still more research needed to determine the appropriate source of MSCs, route and time of administration, and the generation of modified MSCs in which the protective potential is enhanced.

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