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Acute Lung Injury: Disease Modelling and the Therapeutic Potential of Stem Cells

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Abstract

Acute lung injury (ALI) is a severe clinical condition with high morbidity and mortality that usually results in the development of multiple organ dysfunction. The complex pathophysiology of ALI seems to provide a wide range of targets that offer numerous therapeutic options. However, despite extensive studies of ALI pathophysiology and treatment, no effective pharmacotherapy is available. Increasing evidence from both preclinical and clinical studies supports the preventive and therapeutic effects of mesenchymal stem cells (MSCs) for treating ALI. As cell-based therapy poses the risk of occlusion in microvasculature or unregulated growth, MSC-derived extracellular vesicles (MSC-EVs) have been extensively studied as a new therapeutic strategy for non-cell based therapy. It is widely accepted that the therapeutic properties of MSCs are derived from soluble factors with paracrine or endocrine effects, and EVs are among the most important paracrine or endocrine vehicles that can deliver various soluble factors with a similar phenotype as the parent cell. Therapeutic effects of MSCs have been reported for various delivery approaches, diverse doses, multiple origins, and different times of administration, and MSC-EVs treatment may include but is not limited to these choices. The mechanisms by which MSCs and MSC-EVs may contribute to ALI treatment remain elusive and need further exploration. This review provides an overview of preclinical studies that support the application of MSC-EVs for treating ALI, and it discusses emerging opportunities and their associated challenges.

Keywords

Acute lung injury · Animal model · Cell-free therapy · Extracellular vesicles · Intratracheal delivery · Mesenchymal stem cells · Pathophysiology

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Abbreviations

AEC I	Type I alveolar epithelial cells
AEC II	Type II alveolar epithelial cells
ALI	Acute lung injury
Amφ	Alveolar macrophages
ARDS	Acute respiratory distress syndrome
ATS	American thoracic society
BALF	Broncho-alveolar lavage fluid
DMSO	Dimethyl sulphoxide
EBD	Evans blue dye
ELISA	Enzyme-linked immunosorbent assay
ESCRT	Endosomal sorting complex required for transport
H&E	Hematoxylin and Eosin
hAD-MSCs	Human adipose-derived MSCs
hBM-MSCs	Human bone marrow-derived MSCs
HLA	Human leukocyte antigen
hMens-MSCs	Human menstrual blood-derived MSCs
hUC-MSCs	Huma umbilical cord-derived MSCs
i.t.	Intratracheal
i.v.	Intravenous
LPS	Lipopolysaccharide
MFGE8	Milk fat globule-EGF factor 8 protein
MHC	Major histocompatibility complex
MISEV	Minimal information of studies of extracellular vesicles
MPO	Myeloperoxidase
MSC-EVs	MSC-derived extracellular vesicles
MSCs	Mesenchymal stem cells
PARDS	Pediatric ARDS
PDCD61P	Programmed cell death 6 interacting protein
PMN	Polymorphonuclear
qRT-PCR	Qualitative reverse transcriptase polymerase chain reaction
TSG101	Tumor susceptibility gene 101 protein
TSPAN29	Tetraspanin 29
VILI	Ventilator-induced lung injury

1 Introduction

1.1 Acute Lung Injury

Acute lung injury (ALI) or its clinical manifestation, acute respiratory distress syndrome (ARDS), is an acute inflammatory lung injury that usually is responsible for high morbidity and mortality as well as the development of multiple organ dysfunction. ARDS was first proposed in 1967 (Ashbaugh et al. 1967); "A" originally was the abbreviation for adult, but it was later changed to acute. As our understanding of the condition grew, the definition changed from the American-European Consensus Conference Committee definition (Bernard et al. 1994) to the Berlin definition (Force et al. 2012; Ferguson et al. 2012). The latter classifies the severity of the condition from mild to severe. In addition, the pediatric ARDS (PARDS) definition was developed by the Pediatric Acute Lung Injury Consensus Conference in 2015 (Pediatric Acute Lung Injury Consensus Conference Group 2015). Although tremendous progress has been made both in therapy and nursing over the last half century, ALI is still a significant source of morbidity, mortality, and financial burden.

Over three million patients suffer from ARDS every year, and they constitute more than 10% of patients of intensive care units. Moreover, ARDS is likely to be underreported in low-income countries, as it is under-recognized even in high-income countries (Thompson et al. 2017; Villar et al. 2016). Bellani et al. (2016) studied 29,144 patients from 459 intensive care units in 50 countries across 5 continents and found that clinical recognition rates ranged from 51.3% for mild ARDS to 78.5% for severe ARDS, and the condition appeared to be a public health problem globally, with a very high mortality of approximately 40%. Even patients who survive from ALI are at high risk for long-term poor quality of life (Herridge et al. 2016; Biehl et al. 2015). Children are no exception, as another international study that involved 23,280 patients from 145 pediatric intensive care units in 27 countries found that PARDS occurs in approximately 3% of patients

but results in ~17% mortality (Khemani et al. 2019).

To date, there has been no comprehensive epidemiology study of ARDS in China, but the incidence, mortality, and risk factors for ARDS and PARDS in China are thought to be similar to those in Europe and the United States based on several relatively regional studies, which suggest that the annual number of cases in China is more than 670,000 patients (Song et al. 2014). However, health emergency related ALI is not included. During twenty-first century, there are three outbreaks of coronavirus infection around the world, including SARS (Severe Acute Respiratory Syndrome) in 2002, 10 years later with MERS (Middle East Respiratory Syndrome) in 2012 and more recently from December 2019 with COVID19 (Corona Virus Disease). So far, there is no principle to follow for the therapy of COVID19 especially for severe patients because of absence of efficacious drugs and vaccines for SARS and MERS until now. Under the support of WHO (World Health Organization), several specific treatments were under investigation and would be tested through clinical trials, and cell therapy was included.

ALI is a public health problem and common complication in critically ill patient groups, with significantly high mortality and poor outcome. No effective pharmacotherapy exists, so it is necessary to further investigate its pathophysiology and try to find more effective treatments. The goals of this review are to summarize evidence from preclinical studies that supports more efficient therapy for ALI and to discuss emerging opportunities and their associated challenges. The use of mesenchymal stem cells (MSCs) has the potential to treat a variety of diseases, and MSC-derived extracellular vesicles (MSC-EVs) could be a future novel treatment strategy for pulmonary inflammatory disease via intratracheal delivery.

1.2 Pathogenesis of ALI

ALI originates from multiple factors, including direct and indirect lung injury (Table 1). Once triggered by infectious, chemical, or mechanical insult, the complex interaction between the immune system and the alveolar-capillary barrier gives rise to the pathophysiology of ALI (Lee et al. 2019). In addition, genetic studies in Chinese populations identified some genetic risk factors that might increase the development of ARDS, such as Toll-interleukin 1 receptor domain-containing adapter protein (Song et al. 2010) and the tumor necrosis factor receptor-associated factor 6 gene (Song et al. 2012). ALI is also a serious perioperative complication with crucial mortality and morbidity, and there are limited treatments available beyond conservative respiratory support (Jin et al. 2017).

Figure 1 shows the pathogenesis of ALI, including the exudative, proliferation, and fibrotic phases, and the difference between healthy and ALI alveoli (Thompson et al. 2017). The exudative phase usually takes place within 24 h of the

Table 1 Conditions associated with ALI

Direct lung injury insults	Indirect lung injury insults
Pneumonia[a]	Sepsis[a]
Gastric aspiration[a]	Major trauma
Pulmonary contusion	Non-cardiogenic shock
Pulmonary embolism	Pancreatitis
Inhalation injury	Severe burns
Near drowning	Multiple transfusion or transfusion-associated acute lung injury
	Cardiopulmonary bypass surgery
	Reperfusion edema after lung transplantation or embolectomy
	Drug overdose
	Genetic risk factors

[a]Pneumonia, gastric aspiration, and sepsis are the top three main triggers of ALI in recent clinical conditions

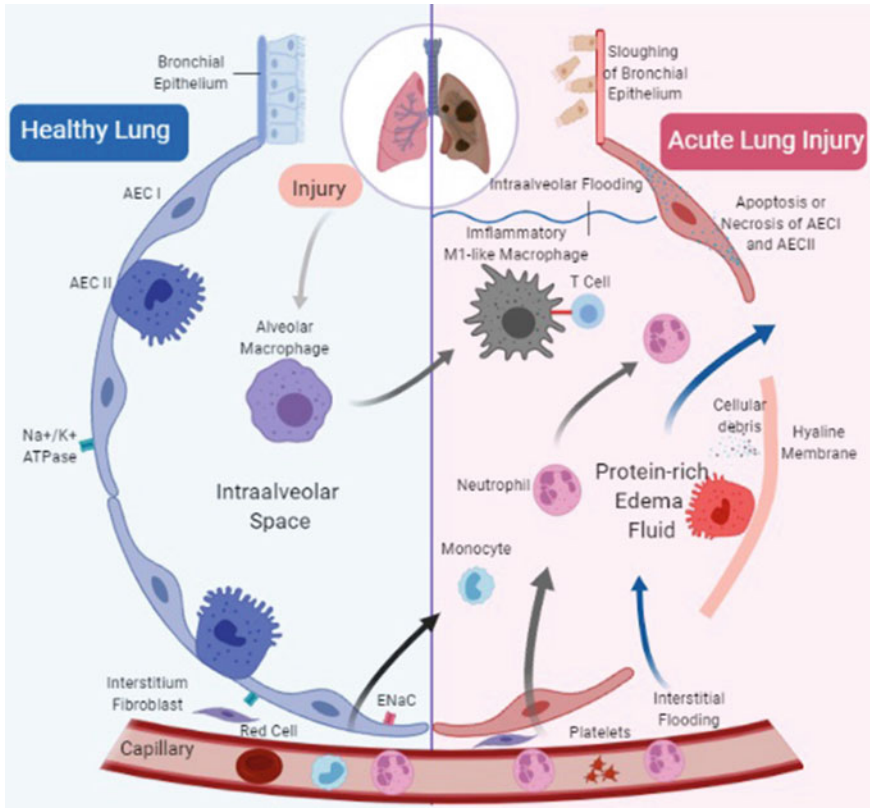

Fig. 1 The difference between a healthy and ALI alveolus. The hallmarks of ALI are disruption of alveolar-capillary barriers, recruitment of pro-inflammatory cells, formation of hyaline membranes, and flooding of protein-rich edema within the interstitium and alveolus. Injury begins with the disruption of alveolar-capillary integrity by either direct or indirect insults. Initially, resident alveolar macrophages are activated and polarized into M1-like macrophages, which secrete pro-inflammatory factors that contribute to recruitment of neutrophils and monocytes to facilitate and maintain inflammation and tissue injury. Extensive damage to the alveolar epithelium directly increases the permeability of alveolar-capillary barriers, and apoptosis of AEC II weakens pulmonary surfactant secretion and alveolar fluid clearance, which aggravate protein-rich edema fluid in the interstitium and alveolus

occurrence of insult; it is characterized by diffuse alveolar damage and represents innate cell-mediated damage of the alveolar endothelial and epithelial barriers and accumulation of protein-rich edema fluid in the interstitium and alveolus. Resident alveolar macrophages (Amφ) recognize microbial components or tissue injury via pattern recognition receptor signaling, which leads to NFκB-dependent polarization of Amφ into M1-like macrophages and the initiation of the exudative phase. M1-like macrophages secrete pro-inflammatory cytokines and chemokines that contribute to accumulation of neutrophils and monocytes as well as to activation of alveolar epithelial cells and effector T cells, which promote and maintain inflammation and tissue injury (Aggarwal et al. 2014). Activated neutrophils contribute to lung injury by releasing pre-formed inflammatory mediators, reactive oxygen species, and proteinases and by the formation of neutrophil extracellular traps and highly injurious histones. The injured and activated endothelium and epithelium initiate tumor necrosis factor-mediated expression of tissue factor, which results in coagulation of dysregulated intra-vascular and intra-alveolar, platelet aggregation, micro-thrombi formation, and hyaline membrane formation. Extensive damage to the alveolar epithelium also leads to the loss of alveolar ion channels and weakens the

osmotic pressure for alveolar fluid clearance, which further promotes alveolar flooding. Endothelial activation and microvascular injury further facilitate alveolar-capillary barrier disruption as well as interstitial and intra-alveolar flooding in ALI. Alveolar flooding and collapse result in severely compromised gas diffusion and hypoxemia.

Impaired and extensive epithelial injury results in the proliferative phase of ALI, which is essential for host survival and is characterized by the transient expansion of resident fibroblasts, the formation of a provisional matrix, and proliferation of airway progenitor cells and differentiation from type II alveolar epithelial cells (AEC II) to type I alveolar epithelial cells (AEC I) (Vaughan et al. 2015). In adult humans, this phase usually occurs between 3 and 7 days following respiratory failure, whereas the timing was reported to be 1 week after injury for experimental animals (Matute-Bello et al. 2011; Beasley 2010). When epithelial integrity has been rebuilt, alveolar edema is reabsorbed and alveolar architecture and function are restored.

The final fibrotic phase does not occur in all ALI patients, but evidence suggests that this phase is related to prolonged mechanical ventilation and increased mortality. Patients in this phase are substantially related to the demand of mechanical ventilation, the development of interstitial and intra-alveolar fibrosis results from extensive basement membrane damage and inadequate or delayed re-epithelialization.

1.3 The Status of ALI Treatment

Lung disease research has shown that ALI is a syndrome characterized by substantial heterogeneity (Thompson et al. 2017). The complex pathophysiology of ALI seems to provide a wide range of targets that offer numerous therapeutic options, but to date there is no available pharmacotherapy based on the pathophysiology of ALI. Currently, ALI treatment is limited to primarily supportive care approaches, such as lung-protective ventilation (Beitler et al. 2016), the fluid conservative strategy (National Heart and B.I.A.R.D.S.C.T. Network 2006), and prone positioning (Fan et al. 2017). Unfortunately, supportive therapies for ARDS focus only on preventing further lung injury rather than actively accelerating tissue repair, and this is why the treatment effectiveness is so limited. In addition, current evidence from practice and research indicates that there is no safe tidal volume or airway pressure for ALI patients. Because the aerated lung volume decreases during the course of the disease, even normal tidal volumes delivered with airway pressure may induce regional overstretch, leading to further epithelium activation or injury and inflammation amplification. For patients who suffer from moderate-to-severe ARDS, ventilation while in the prone position is closely associated with decreased mortality, and this is currently recommended in clinical practice (Fan et al. 2017). Unfortunately, no pharmacologic therapy has been shown to decrease ARDS either short-term or long-term mortality. Therefore, new approaches to develop feasible therapies for ALI are needed.

2 Animal Models to Study ALI

2.1 Experimental Animal Models of ALI

Animal studies provide an experimental scenario that allows investigators to study underlying pathophysiological mechanisms and search for therapeutic approaches before translating them into humans. A good animal model should share similar anatomy and responses with humans so that it can be used to predict the feasibility of a therapeutic approach and provide a bridge from bench to bedside. However, no animal model can perfectly duplicate all human features when exposed to stimuli or treatments, and the ALI animal model is no exception. As recommended in the official documents of the American Thoracic Society (ATS), at least three of four main features of ALI should be present in an ALI animal model, including histological evidence of tissue injury, alteration of the alveolar capillary barrier, inflammatory response, and physiological

dysfunction, which are specifically described in Table 2 (Matute-Bello et al. 2011). ATS documents also indicate that it is not necessary to establish a fully developed ALI animal model. In this regard, we previously summarized cellular mechanisms underlying lung regeneration and repair, and analyzed the role of stem cells both in small and large animal models (Yahaya 2012).

2.2 Evaluation of Common ALI Animal Models

For preclinical studies, numerous methods to develop animal models for ALI have been reported, including endotoxin (Wang et al. 2018; Zhu et al. 2017; Tang et al. 2017), bacteria (Monsel et al. 2015), ventilator (Hayes et al. 2015; Islam et al. 2019), and cecal ligation and puncture (Wang et al. 2015; Condor et al. 2016). According to these studies, neutrophils play an important role in the inflammatory response in ALI development in animal models, both for small and large animal.

Most ruminants, including goats and sheep, have segmented lungs, which means that many macrophages circulate in pulmonary vessels and that their pulmonary circulation tends to be sensitive to intravenous injection with endotoxin. Several studies reported that small doses of endotoxin induced increased pulmonary hypertension in these animals, and previous studies showed that smoke inhalation injury (Lange et al. 2012; Rehberg et al. 2013; Halim et al. 2019) and brushing injury (Yahaya et al. 2011; Kardia et al. 2018). Infiltration and accumulation of neutrophils were also reported to be the major feature in large animal models for ALI (Lange et al. 2012), but large animals are prone to microbial infection, so intravascular macrophages in these animals are easily augmented via stimulation of the local inflammatory reaction in response to microbe invasion.

In contrast, smaller animals and humans have fewer intravascular macrophages. Compared with large animals, small animals such as mice, rats, and rabbits are widely bred and very economical in terms of expenses. Numerous studies indicated that endotoxin-induced ALI in mice resulted in prominent inflammatory cell infiltration in the alveolar spaces, including neutrophils and macrophages, as well as interstitial edema and intra-alveolar septal thickening with fibrin and collagen deposition (Chen et al. 2014; Liou et al. 2017). The rat model showed a similar pattern of ALI characteristics following exposure to toxic chemicals such as sodium nitrate and naphthalene (Uriarte et al. 2013; Zhang et al. 2016). Moreover, activated neutrophils were found to play a key role in initiating the inflammatory processes involved in the formation of hemorrhage or alveolar damage (Wang et al. 2018; Zhu et al. 2017; Zhang et al. 2018). Murine lungs rarely develop hyaline membranes following ALI (Matute-Bello et al. 2011), whereas hyaline membranes in rabbit ALI models usually appear during the early exudative phase of ALI (Cao et al. 2012), which is consistent with the features in ALI patients. Moreover, gene sequence comparison analysis demonstrated that the rabbit shared a higher homology with the human leukocyte antigen (HLA) genes than mouse and rat, thus rabbit tissue is less likely to result in immune rejection of allotransplantation (Marche et al. 1985). Therefore, rabbits are commonly used in implantation and tissue engineering studies. However, the greater availability of specific reagents and genetically modified mice and rats make them popular for animal models.

The official ATS workshop report recommends the features and measurements of experimental ALI animal models and also describes the difference between ALI patients and several common ALI animal models in detail (Matute-Bello et al. 2011). Given the high frequency of use, we briefly summarized LPS, ventilator, and live bacteria-induced lung injury in Table 2. Almost all of the "very relevant" criteria are present in the top three most common animal models, so they can be used to further investigate the more efficient therapeutic approaches to treating ALI. The LPS-induced ALI mouse model is commonly used as a model of human ALI associated with severe pneumonia or sepsis because of its high efficiency. Intratracheal (i.t.) and intravenous (i.v.) delivery are commonly

Table 2 Presence of "very relevant" criteria in the top three common animal models of ALI

Human patient		Measurement	Notes	LPS	VILI	Live bacteria
Histological evidence of tissue injury	Accumulation of neutrophils in the alveolar/interstitial space	H&E staining	Hyaline membranes are rarely observed in murine models	+	+	+
	Formation of hyaline membranes			+	+	+
	Proteinaceous debris in alveolar space			+	+	+
	Thickening of the alveolar wall			+	+	+
	Injury by a standardized histology score			+	+	+
Alteration of the alveolar capillary barrier	Increased extravascular lung water content	Wet-to-dry ratios	More errors for very small lungs	+	+	+
	Accumulation of protein/tracer in airspaces/extravascular space	EBD	Intravenous injection in advance	+	+	+
	Total BAL protein concentration	BALF-total protein concentration, IgM	Technical challenges and difficult to standardize	+	+	+
	BAL concentration of high molecular weight proteins			+	+	+
	(Micro-)vascular filtration coefficient (K_f)	Under machine testing	Only for isolated perfused lung	(+)	+	(+)
Inflammatory response	BAL total neutrophil counts	BALF-cytospin, Wright-Giemsa staining	Neutrophil number and percentage	+	+	+
	Lung MPO activity	ELISA kits or colorimetric assay	Cell-free BALF or whole lung homogenates	+	+	+
	Concentrations of cytokines	qRT-PCR or ELISA kits	mRNA or protein expression	+	(+)	+
Physiological dysfunction	Hypoxemia	Under machine testing	Equipment limits	+	+	+
	Increased alveolar-arterial oxygen difference			+	+	+

Notes: *LPS* lipopolysaccharide, *VILI* ventilator-induced lung injury, *BALF* broncho-alveolar lavage fluid, *EBD* Evans blue dye, *MPO* myeloperoxidase, *RT-PCR* reverse transcriptase polymerase chain reaction, *H&E* Hematoxylin and eosin, *ELISA* enzyme-linked immunosorbent assay

+, the criterion was present in virtually all studies using this model

(+) the criterion was present in the majority of studies using this model

used to induce ALI in animal models in preclinical studies, but there are some differences between these two delivery approaches. The former is pulmonary administration, and the ALI model reveals how the alveolar epithelium structure in the lungs is injured, including by polymorphonuclear (PMN) cell infiltration in intra-alveolar areas, diffuse alveolar edema, and mild changes in epithelial permeability. Use of i.v. delivery shows how the vascular endothelium structure in the lungs is injured, such as via PMN cell accumulation in capillaries and the interstitium with mild infiltration in intra-alveolar areas, presence of protein-rich alveolar edema, and mild changes in epithelial permeability. Just like in human patients, i.t. and i.v. delivery in animal models mimic direct and indirect insult, respectively. However, these animal models usually heal with few areas of fibrosis remaining, so investigators should adjust the time points in

preclinical studies, especially in therapeutic research (Lopes-Pacheco et al. 2019).

3 Stem Cell Therapy for ALI

Cell therapies are new potential treatments that aim to repair injured tissue and mitigate inflammation via regeneration by virtue of their multipotency as well as the release of and regulation by their soluble bioactive factors. In recent years, preclinical studies have shown the potential of cell therapies in treating lung diseases and critical illness, and they are likely to provide novel therapeutic candidates for general ARDS patients. In this context, our group has previously established an aerosol-based cell therapy using AECs for the treatment of ALI models, both in vivo and in vitro. The results indicated that AEC delivery remarkably accelerated the repair and regeneration of the respiratory airway (Kardia et al. 2017, 2018). Currently, adult stem cells have been regarded as a promising approach for ALI because of their ability to alleviate the major pathologies underlying ALI (Zhu et al. 2013). Stem cell therapy for ALI disease is recognized as a promising option not only for controlling symptoms but also for its potential benefit as a curative treatment regimen.

3.1 Cell Therapy Using MSCs

To date, MSCs may offer the best choice for clinical trials due to their multi-lineage differentiation capability, potent ability to modulate the inflammation process and immune system, diverse sources, ease of harvesting, and extensive preclinical studies (Kim and Park 2017). MSCs are non-hematopoietic multipotent stem cells derived from a variety of tissues such as bone marrow, adipose tissue, umbilical cord, placenta, dental pulp of deciduous baby teeth, menstrual blood, and several organs including the liver, spleen, and lung (Samsonraj et al. 2017). In vitro functional studies indicate multiple physiological roles of MSCs related to their heterogeneity and tissue location of origin (Sacchetti et al. 2016; Klimczak and Kozlowska 2016; Heo et al. 2016). The International Society of Cellular Therapy has defined MSCs as cells having these criteria: (1) They adhere to a plastic surface under standard tissue culture conditions; (2) they express certain cell surface markers, such as CD73, CD90, and CD105, but they must not express other markers, including CD45, CD34, CD14 or CD11b, CD79a, CD19, and HLA-DR; and (3) they are able to differentiate into osteoblasts, adipocytes, and chondroblasts under standard in vitro conditions (Dominici et al. 2006). Numerous studies have demonstrated the therapeutic potential of MSCs in multiple diseases, especially for tissue injury and degenerative and immunological diseases.

Halim et al. (Halim et al. 2019) previously investigated the effects of MSC treatment on asthma-related airway inflammation via aerosolization delivery, and the results demonstrated that MSC treatment relieved airway inflammation and reversed airway remodeling. Additionally, MSCs likely are able to elude clearance by the host immune system through a variety of mechanisms, including low expression of MHC I and II proteins and lack of the T-cell costimulatory molecules, CD80 and CD86; thus, they often are referred to as being ‘immuno-privileged’ (Lee et al. 2011). Past studies provide a powerful basis for exploring innovative approaches for the treatment of inflammatory diseases.

Several pilot clinical trials were conducted by research institutes or hospitals from all over the world, and they can be tracked on ‘clinical trial.gov’ (Table 3). The aim of most of the clinical trials was to assess the safety and efficiency of MSCs in patients with ALI/ARDS. Only three early-stage clinical trials have been completed with updated results, and they demonstrated that one dose of MSCs with intravenous delivery was safe for moderate to severe ARDS patients (Zheng et al. 2014; Wilson et al. 2015; Matthay et al. 2019). However, there are multiple challenges for evaluation of treatment efficiency, as dosage, time interval, delivery route, and illness severity must be considered and compared between MSC-treated and placebo groups. Currently, the optimum therapeutic dosage of MSCs

Table 3 Clinical trials of MSC-based therapies for ALI/ARDS patients

No.	ID	Phase	Treatment	Intervention	Enrollment	Follow-up	Status	Results	Country	References
1	NCT01902082	I	hAD-MSCs	1 million cells/kg, single dose, i.v.	12 (6/6)	28 days	Completed	MSC administration is safe and well tolerated, but significant difference on clinical outcome was weak	China	Zheng et al. (2014)
2	NCT01775774	I	hBM-MSCs	Dose-escalation: 1/5/10 million cells/kg, single dose, i.v.	9 (3/3/3)	12 months	Completed	All MSC dose levels were well tolerated, with no infusion-related adverse events	USA	Wilson et al. (2015)
3	NCT02097641	II a	hBM-MSCs	10 million cells/kg, single dose, i.v.	60 (40/20)	12 months	Completed	A trend for improvement in oxygenation index was observed in the MSC group, but it was not significant.	USA	Matthay et al. (2019) Extension of NCT01775774
4	NCT02804945	II	hBM-MSCs	3 million cells/kg, single dose, i.v.	20	60 days	Completed	No results posted	USA	No reference available
5	NCT02611609	I/II	MultiStem	Low/high dose, no details	36	12 months	Completed	No results posted	USA/UK	No reference available
6	NCT03608592	Not applicable	hUC-MSCs	1 million cells/kg, single dose, i.v.	26	60 days	Recruiting	No results posted	China	No reference available
7	NCT02444455	I/II	hUC-MSCs	0.5 million/kg, once daily for 3 days. i.v.	20	14 days	Unknown	No results posted	China	No reference available
8	NCT02095444	I/II	hMens-MSCs	10 million cells/kg, twice a week for 2 weeks, i.v.	20	14 days	Unknown	No results posted	China	No reference available
9	NCT02112500	II	hBM-MSCs	i.v., no details	10	28 days	Unknown	No results posted	Korea	No reference available
10	NCT03042143	I/II	hUC-MSCs	Dose-escalation: 100/200/400 million cells/patient, single dose, i.v.	75	28 days	Recruiting	No results posted	UK	No reference available
11	NCT03807804	II	hBM-MSCs (MultiStem HLCM051)	900 million cells/patient, single dose, i.v.	30	28 days	Recruiting	No results posted	Japan	No reference available

(continued)

Table 3 (continued)

No.	ID	Phase	Treatment	Intervention	Enrollment	Follow-up	Status	Results	Country	References
12	NCT02215811	I	hBM-MSCs	Not reported	10	12 months	Unknown	No results posted	Sweden	No reference available
13	NCT03552848	Not applicable	hUC-MSCs	1 million cells/kg, once every 4 days for 4 times, i.v.	60 (30/30)	24 months	Recruiting	No results posted	China	No reference available
14	NCT03818854	II b	hBM-MSCs	10 million cells/kg, single dose, i.v.	120 (60/60)	60 days	Not yet recruiting	No results posted	USA	Extension of NCT01775774 & NCT02097,641

All information was taken from: https://clinicaltrials.gov/

Notes: *MSCs* mesenchymal stem cells, *BM* bone marrow-derived, *UC* umbilical cord-derived, *AD* adipose-derived, *Mens* menstrual blood-derived, *h* human, *i.v.* intravenous

for treating lung diseases is unknown. In addition, and perhaps more importantly, we do not know whether it is necessary to deliver multiple doses of MSCs to treat advanced ALI animal models or ARDS patients.

Although the precise therapeutic mechanisms by which MSCs alleviate ALI remain unclear, a number of important insights from recent preclinical studies have emerged (Fig. 2), and they include but are not limited to cell-to-cell interactions and secretion of soluble factors, such as growth factors, matrix proteins, cytokines and extracellular vesicles, as well as through mitochondrial transfer (Lee et al. 2019; Lopes-Pacheco et al. 2019; Zhu et al. 2013; Abraham and Krasnodembskaya 2019). MSCs have been proven to play crucial roles in anti-inflammatory and anti-apoptotic activities, to facilitate epithelial and endothelial cell restoration, and to increase microbial and alveolar fluid clearance, resulting in the improvement of lung and distal organ injury as well as survival (Lopes-Pacheco et al. 2019; Xiang et al. 2017; Pedrazza et al. 2017; Morrison et al. 2017; Ren et al. 2018).

In support of these findings, Halim et al. (Halim et al. 2018) demonstrated that MSC-secreted proteins facilitated airway epithelial repair by stimulating the regenerative ability and endogenous reparation of lung cells, and most of proteins were extracellular proteins. In addition, MSCs have been demonstrated to alleviate LPS-induced ALI through downregulation of miR-142a-5p, which mediates autophagy of pulmonary endothelial cells by increasing Beclin-1 protein (Zhou and You 2016). Additionally, the NF-κB, MAPK, and STAT3 signaling pathways are all thought to be involved in the effects of MSC treatment in the ALI animal model, but more studies are needed to elucidate the therapeutic mechanism. So far, our group has already explored the feasibility of cell therapy both in chronic (Halim et al. 2019) and acute lung disease (Kardia et al. 2018); the results were consistent with each other, which showed

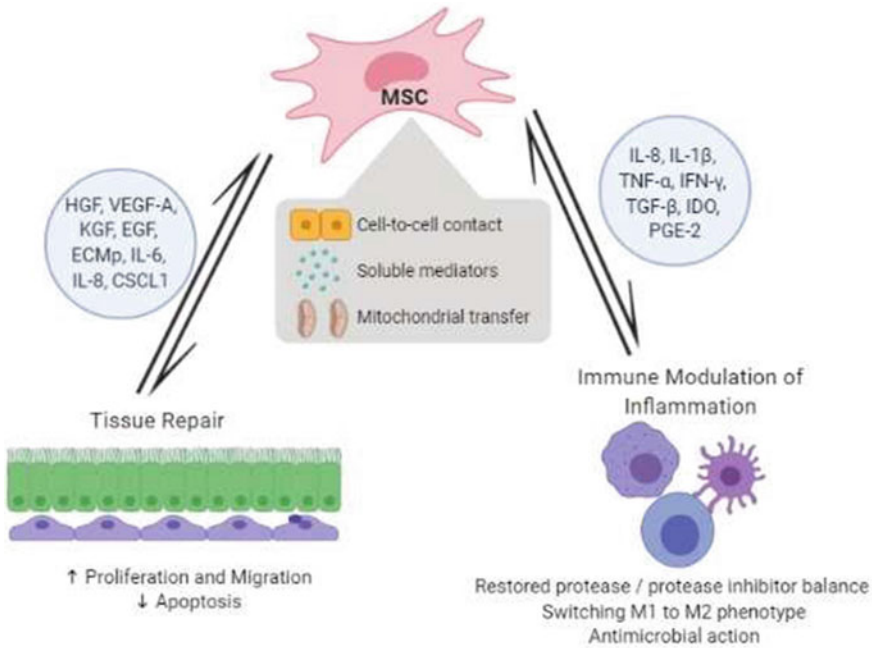

Fig. 2 Mechanisms underlying the modulation of inflammation and lung tissue repair by MSCs in ALI. MSCs have been proven to facilitate tissue repair and relieve inflammation by cell-to-cell contact, mitochondrial transfer, and paracrine or endocrine soluble factors, including growth factors, anti-inflammatory cytokines, and chemokines, as well as EVs

that MSCs or AECs reduced inflammation of the lung and airways and facilitated lung regeneration. In summary, all of these studies provided essential knowledge and data to support the therapeutic potential of MSCs to treat ALI conditions.

Nonetheless, cell-based therapy poses the risk of occlusion in microvasculature and unregulated growth in vivo. Among these concerns, the first is the risk of tumor formation. Additionally, extensive ex-vivo expansion are required for sufficient cell numbers in clinical protocols. Controversies about the stability of human derived MSCs (hMSCs) highlight the need to address hMSC stability in long-term cultures before use in clinical treatment (Bernardo et al. 2007; Meza-Zepeda et al. 2008; Rosland et al. 2009). A numbers of studies indicated that hMSCs may contribute to cancer development and progression either by acting as cancer-initiating cells or through interactions with stromal elements (Herberts et al. 2011). Lee and Hong (2017) demonstrated that MSCs have the ability to accelerate tumor growth due to their ability to migrate and home to the tumor site and alter its microenvironment, and they also can produce cytokines that stimulate tumor growth. However, although MSCs have the ability to induce tumor growth, there is no evidence from MSC clinical trials showing the involvement of MSCs in tumor development. Further research is needed before MSCs can be considered as a safe candidate for clinical treatment in patients.

Another issue related to MSC use is dose optimization in terms of number of cells in a single dose for testing in both preclinical and clinical experiments. High doses of MSCs are associated with several safety concerns; for example, a high dose by i.v. delivery could induce pulmonary embolism. Finally, MSCs are live cells, so particular care must be taken in their storage and transportation. DMSO is required as a preservative for MSC cryopreservation, and the process of cryopreserving and thawing reduce the viability of MSCs, which could have an adverse effect on their therapeutic efficacy in patients (Matthay et al. 2019). In view of these issues, there is an urgent need to find a safer cell-free therapeutic approach. It is widely accepted that the paracrine effects of MSCs are due mainly to mediation by extracellular vesicles (EVs) secretion, so the therapeutic potential of MSC-derived EVs is being actively explored as an alternative approach to MSC-based treatments.

3.2 MSC-EVs as a Potential Therapeutic for ALI

Initially, the therapeutic effects of MSCs were thought to derive from their engraftment in the injury site and regeneration afterwards. However, subsequent experimental evidence demonstrated that most MSCs administered get trapped in capillary networks and are transient in injury sites, which indicates that engraftment plays little role in therapeutic action (Eggenhofer et al. 2014). Subsequent studies demonstrated that the therapeutic properties of MSCs are derived from soluble factors with paracrine or endocrine effects, including growth factors, anti-inflammatory cytokines, and antimicrobial peptides, which can facilitate alveolar epithelial proliferation and stabilize the alveolar-capillary barrier, regulate the inflammatory microenvironment, and enhance alveolar fluid clearance and decrease infection (Lee et al. 2011). These findings provided a sufficient theoretical basis for the usage of novel cell-free therapies. MSC-EVs would be one of the most compelling alternatives for cell-free therapy because of their lower risk of allogenic immune rejection, accessible preservation, and higher stability compared with MSCs. In addition, EVs can carry micro and messenger RNAs as well as lipids, proteins, and even organelles, which can be used to regulate the behavior of target cells and shift gene expression (Yáñez-Mó et al. 2015). EVs also can bypass the blood-brain barrier by transcytosis through the endothelial layers to deliver cargo biomolecules to the brain parenchyma (Chen et al. 2016). The therapeutic application of MSC-EVs remains promising, and recent studies have underlined the new potential role of EVs as a paracrine of endocrine vehicle to deliver multiple soluble factors with a similar phenotype as the parent cell (Zhu et al. 2014).

Additionally, compared to their parent cells, EVs can be safely stored without losing function.

EVs were first clearly described by Pan and Johnstone (1983). Initially, EVs were thought to be a disposal mechanism by which cells eliminate unwanted proteins and other molecules. Subsequent research showed that EV secretions are important mediators of cell-to-cell communication that is involved in normal physiological process but also plays a crucial role in the development and progression of diseases. EVs are classified based on their cellular origin, biological function, and biogenesis, and the three main classes recognized currently are exosomes, microvesicles, and apoptotic bodies (Table 4) (El Andaloussi et al. 2013). According to the guidelines from the International Society for Extracellular Vesicles' minimal information for studies of extracellular vesicles 2018 (MISEV2018), EVs can be characterized by four distinct aspects (Théry et al. 2018). The first is quantification of EVs, both in terms of number of cell sources and the amount of EVs from a given number of cells; measurements can include the total levels of protein, lipids, or RNA. This aspect suggests that it would be informative to analyze at least one membrane bound (CD63, CD9, or CD81) and one cytosolic protein (TSG101 or ALIX) in EVs. The secondly aspect is the protein composition, specific markers in proteins, and non-protein components of EVs, and these can be analyzed using Western blotting or PCR (Hartjes et al. 2019). Third, single vesicle analysis of EVs can be conducted using visualization techniques such as transmission electron microscopy (Shao et al. 2018). Finally, other EV-associated components can be evaluated by topology analysis.

Importantly, Ratajczak et al. (2012) reported that EVs secreted by stem cells contributed to their maintenance and plasticity; in other words, stem cell-derived EVs play a critical role in tissue regeneration after injury. For example, EVs from MSCs have been used to stimulate tissue repair following cardiovascular (Lai et al. 2011), kidney (Bruno et al. 2016; Song et al. 2017) and lung (Lee et al. 2011; Hayes et al. 2012; Monsel et al. 2016) injury. On the other hand, the effect of EVs on regulation of the immune response depends on the status of particular immune cells, as they might trigger adaptive immune responses or suppress inflammation in a tolerogenic manner (Robbins and Morelli 2014). Such wide-raging cellular and biological functions indicate that MSC-EVs, in virtue of their pleiotropic signaling, may have innate therapeutic potential for regenerative medicine and immunotherapy. Moreover, Phinney et al. (2015) reported that there are functionally active mitochondria and numerous miRNAs in MSC-EVs. This is an important finding because ALI always clinically results in multiple organ dysfunction syndrome, which is

Table 4 Classification and characterization of EVs

Types	Origin	Size	Content	Markers
Exosomes	Endolysosomal pathway; intraluminal budding of multivesicular bodies and fusion of multivesicular body with cell membrane	40–120 nm	mRNA, miRNA, and other non-coding RNAs; cytoplasmic and membrane proteins including receptors and MHC molecules	Tetraspanins (TSPAN29 and TSPAN30), ESCRT components, PDCD61P, TSG101, flotillin, MFGE8
Microvesicles	Cell surface; outward budding of cell membrane	50–1,000 nm	mRNA, miRNA, non-coding RNAs, cytoplasmic proteins, and membrane proteins, including receptors	Integrins, selectins, CD40 ligand
Apoptotic bodies	Cell surface; outward blebbing of apoptotic cell membrane	500–2000 nm	Nuclear fractions, cell organelles	Extensive amounts of phosphatidylserine

Notes: *ESCRT* endosomal sorting complex required for transport, *MFGE8* milk fat globule-EGF factor 8 protein, *PDCD61P* programmed cell death 6 interacting protein (also known as ALIX), *TSG101* tumor susceptibility gene 101 protein, *TSPAN29* tetraspanin 29

associated with mitochondrial dysfunction. Therefore, mitochondria-targeted strategies are increasingly being explored as a promising therapeutic approach for treating lung injury, and MSC-EV-mediated mitochondria transfer is one of the most exciting among them (Agrawal and Mabalirajan 2015).

Although preclinical studies of the therapeutic use of MSCs-EVs in ALI are still in their infancy, MSCs-EVs are thought to be as powerful as their parent cells in promoting remission in ALI and other inflammatory lung disease models because they pass their cargo to recipient cells, thus facilitating the therapeutic benefits. The application potential of EVs versus intact live cells is significant because: (1) they have no iatrogenic tumor risk because of their non-self-replicating property; (2) they can be stored at −80 °C without DMSO and without loss of biological activity; (3) they offer potential for multiple doses or a higher single dose without significantly affecting the patient's hemodynamic or respiratory variables; and (4) they do not express MHC antigens so they can be used for allogeneic transplantation. However, utilization of MSC-EVs will require large-scale production and standardization, which pose issues concerning identification, characterization, and quantification.

3.3 Routes of Cell Delivery and Their Therapeutic Impacts

The therapeutic benefits of MSCs for ALI summarized all relevant articles from 2007 to 2019 for both natural and modified/preconditioned MSCs, and it demonstrated that many ALI models involved LPS by i.t. and i.v. challenge (Lopes-Pacheco et al. 2019). The preclinical studies of MSCs in ALI used various delivery approaches (systemic or local), diverse doses, multiple origins (bone marrow, umbilical cord, menstrual blood, adipose, or other tissues), and different schedules of administration (before or after challenge). However, bone marrow and umbilical cord are the more common sources of MSCs, and umbilical cord-derived MSCs are currently most popular for clinical application due to their accessibility and lack of ethical concerns (Li et al. 2012; Sun et al. 2011).

Several studies demonstrated the therapeutic efficacy and mechanism of action of human MSCs in a mouse ALI model induced by i.t. administration of LPS; i.v. delivery at 4 h (Zhang et al. 2018; He et al. 2015; Xu et al. 2018; Liu et al. 2018) and 6 h (Ren et al. 2018) after LPS challenge; or i.t. delivery at 4 h post-LPS challenge (Wang et al. 2018; Ionescu et al. 2012). In these studies, MSCs reduced alveolar inflammation and edema by decreasing the influx of inflammatory cells and total protein levels in the endotoxin-damaged alveolus. In addition, the therapeutic effects of the MSCs were comparable regardless of route of administration. Zhu et al. (2014) used the endotoxin-induced ALI mouse model to explore the therapeutic potential of MSC-EVs in ALI and reported that MSC-EVs decreased the influx of total inflammatory cells into the lung by 36% and the influx of neutrophils by 73%. Similarly, in another mouse model of hypoxia-induced pulmonary hypertension, i.v. injection of MSCs-EVs resulted in delayed pulmonary influx of macrophages and reduced production of pro-inflammatory mediators compared to injection of mouse lung fibroblast-derived EVs (Lee et al. 2012).

MSC administration has been performed via either systemic or local routes in experimental models. Systemic administration (e.g., i.v.) is readily available in clinical practice and provides wide distribution throughout the whole body, but it also results in cell waste along the route. In contrast, local administration (e.g., i.t.) delivers cells to the lung directly, so this is the more straightforward route for treating lung disease. However, i.t. intubation is a more difficult technique, especially for small animals such as mice and rats, whereas the i.v. route is more easily accessible for animals. Although the pulmonary first-pass effect has been detected with i.v. administration (Fischer et al. 2009), which results from cell retention in the lung, this effect may be beneficial for lung repair.

4 Conclusions

In conclusion, ALI remains a severe clinical condition with high morbidity and mortality, both for adults and children, but no effective pharmacotherapy exists. However, MSC-EV therapy is likely to provide a promising option not only for controlling symptoms but also for its potential benefit as a curative treatment regimen. EVs can be readily isolated from MSCs from various sources, and MSC-EVs have shown prominent therapeutic benefits in a range of ALI animal models. MSC-EVs also are considered to be non-immunogenic and break through the blood-brain barrier, so the therapeutic potency of MSC-EVs may be even better compared with their parental cells.

However, the dose, route, and time points of MSC-EV treatments vary substantially based on different preclinical animal studies, and the optimal treatment remains to be determined. Among the various delivery routes, intratracheal instillation seems to be the most straightforward approach to enhancing bacteria clearance, but for practical reasons it may not be feasible to instill MSCs or MSC-EVs for ALI patients (e.g., those who are hypoxemic). Thus, we need to balance efficiency and utility. Intratracheal intubation is the key technique for the pulmonary-induced ALI animal model, and it must be verified before real animal experiments begin. Moreover, many issues need to be addressed before translation of MSC-EVs to clinical trials, including the standardization of MSC-EVs collection, appropriate assessments for MSC-EVs in ALI, and whether we need to extract one or two components from EVs. Nevertheless, MSC-EVs have great therapeutic potential for treating ALI, and this cell-free therapy should be studied further.

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