



Extracellular Vesicles in ARDS: New Insights into Pathogenesis with Novel Clinical Applications

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4.1 Introduction

Acute respiratory distress syndrome (ARDS) is an inflammatory disorder of the lungs that can develop following various insults, the commonest being pneumonia. Patients develop acute hypoxemic respiratory failure following inflammatory injury to the alveolar epithelium and endothelium [1]. The precipitating injury can be direct (e.g., pneumonia, aspiration) or indirect (e.g., peritonitis, pancreatitis, shock). Our understanding of ARDS pathogenesis has increased in the 50 years since this syndrome was first described; however, there are many aspects which continue to elude us [1]. Following the same insult, why do some patients develop ARDS and others do not? How can we predict which critically ill patients are at higher risk of developing ARDS? Advances in ventilation strategies [2] and fluid management have helped to reduce mortality; however, this still remains unacceptably high at 35–45% [3].

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Despite numerous clinical trials, there is still no effective pharmacotherapy available for ARDS patients. However, the rapidly developing field of extracellular vesicles may provide a major new opportunity for an improved understanding of ARDS pathogenesis, valuable biomarkers of injury, and targets for new therapies.

Extracellular vesicles are anuclear structures released by cells and bounded by a phospholipid bilayer membrane. Cells can release extracellular vesicles during states of health, injury/activation, and apoptosis. Extracellular vesicles can contain diverse cargo, including messenger RNA (mRNA), micro RNA (miR), cytokines, and mitochondria. Surface markers on extracellular vesicles can indicate the parent cell from which they were derived, and also determine which cells can incorporate specific extracellular vesicles. We are now beginning to appreciate the role extracellular vesicles play in intercellular communication in health and disease states, facilitating transfer of genetic material, proteins, and organelles between cell types. Distinctions between the two main subtypes of extracellular vesicles are based on size and origin: exosomes are smaller (<150 nm diameter) and may have an endosomal origin. Microvesicles are larger (up to 1 μm diameter) and derived from the cell membrane.

In this chapter, we highlight recent translational and clinical studies that have deepened our understanding of the role extracellular vesicles play in both mediating and attenuating inflammatory lung injury in ARDS. We discuss what future directions can be taken to utilize extracellular vesicles as diagnostic or prognostic biomarkers, as targets for novel therapeutics, or as therapeutic agents in their own right (Fig. 4.1).

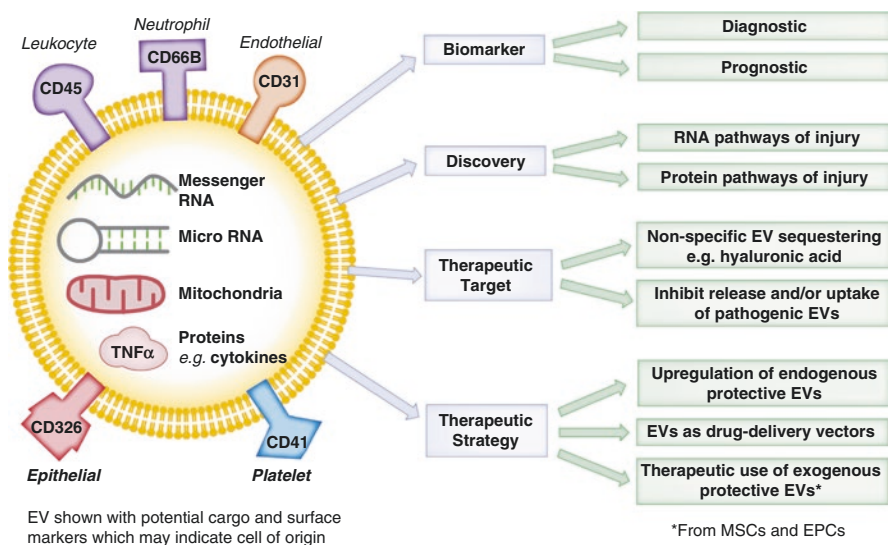


Fig. 4.1 Clinical relevance and applications of extracellular vesicles in acute respiratory distress syndrome (ARDS). Representative extracellular vesicle (EV) shown with phospholipid bilayer, surface markers, and cargo. Surface markers indicate cell of origin (e.g., CD45⁺/CD66b⁺ indicates a neutrophil-derived extracellular vesicle, CD31⁺ endothelial-derived, CD41⁺ platelet-derived, and CD326⁺ epithelial-derived). Surface markers from different cell types shown for illustrative purposes; extracellular vesicles will not concurrently express all surface markers shown. Extracellular vesicle cargo can include micro RNA, messenger RNA, mitochondria, and protein (e.g., cytokines). *EPC* endothelial progenitor cell, *MSC* mesenchymal stem cell, *TNF- α* tumor necrosis factor- α

4.2 Contribution of Extracellular Vesicles to the Pathogenesis of ARDS

Preclinical models of ARDS have shown that extracellular vesicles released following lung injury can mediate inflammation and have an injurious effect. Endothelial injury is often the earliest pathological event leading to the development of ARDS [4]. Circulating endothelial and leukocyte-derived extracellular vesicles are elevated in the intratracheal lipopolysaccharide (LPS) rat model of lung injury [5]. Human endothelial cells also release extracellular vesicles following stimulation with plasminogen-activated inhibitor-1 [6]. Several studies have reported that intravenous administration of endothelial extracellular vesicles in rodents induced lung injury with alveolar neutrophilic infiltration, pulmonary edema, elevated inflammatory cytokines (myeloperoxidase [MPO], interleukin [IL]-1 β and tumor necrosis factor [TNF]- α), and increased lung endothelial permeability [6–8]. These changes were similar to those observed following intratracheal LPS injury. Endothelial extracellular vesicle treatment of murine or human arterioles impaired nitric oxide release and vasodilation, which partly explains the *in vivo* findings [6]. Endothelial extracellular vesicles administered concurrently with LPS (intratracheal or intravenous) caused a greater increase in alveolar endothelial permeability and inflammatory cytokine release than either LPS or extracellular vesicles alone [6, 7]. However, when endothelial extracellular vesicles were administered 6 h prior to intravenous LPS, the resulting circulating and alveolar inflammatory cytokine release was significantly greater than with concurrent administration of extracellular vesicles and LPS. An initial endothelial injury triggered release of endothelial extracellular vesicles, which then primed the lung for a greater inflammatory response when exposed to a subsequent infectious insult.

Following LPS treatment, human endothelial cells release extracellular vesicles containing nitrated sphingosine-1-phosphate receptor-3 (S1PR3) [9]. Elevated circulating S1PR3 concentrations are associated with mortality in critically ill sepsis patients, with or without ARDS. Endothelial extracellular vesicles could therefore represent a potential biomarker and/or offer a novel therapeutic target for ARDS. Simvastatin treatment given concurrently with intravenous LPS in mice reduced endothelial extracellular vesicle release and lung endothelial permeability [10]. This is a particularly interesting finding, since a secondary analysis of an ARDS trial showed that simvastatin reduced mortality in patients with a hyper-inflammatory endotype, suggesting that statin therapy was working in part by inhibiting extracellular vesicle release and lung injury [11]. Therefore, therapies aimed at reducing or blocking endothelial extracellular vesicles may attenuate lung injury.

Recently, the results of studies in the *ex vivo* perfused human lung model have provided compelling new evidence for the potential role of extracellular vesicles in mediating lung injury in ARDS. In an *ex vivo* perfused human lung model of Gram negative pneumonia, injury with intrabronchial *Escherichia coli* led to release of extracellular vesicles by lung tissue into the perfusate [12]; these extracellular vesicles were predominantly endothelial- and platelet-derived. Administration of *E. coli*-induced extracellular vesicles either into the perfusate or into the air spaces in naïve, uninjured human lungs induced injury similar to the degree of lung injury with *E. coli* pneumonia: pulmonary edema, impaired alveolar fluid clearance,

neutrophilic infiltration, and elevated bronchoalveolar lavage fluid (BALF) TNF- α . *E. coli*-induced extracellular vesicles contained high levels of TNF- α and IL-6 mRNA, which explained at least part of their pro-inflammatory effects. Monocyte uptake of *E. coli*-induced extracellular vesicles resulted in increased secretion of TNF- α and IL-6. High molecular weight hyaluronic acid bound CD44 on the surface of *E. coli*-induced extracellular vesicles, thus preventing their uptake by monocytes. Intravenous administration of high molecular weight hyaluronic acid to *ex vivo* human lungs injured with *E. coli* or *E. coli*-induced extracellular vesicles attenuated lung injury, pulmonary edema, BALF TNF- α levels, and histologic evidence of lung injury. This important study showed that following infectious injury, human lung tissue releases pathogenic extracellular vesicles, which can mediate more severe inflammatory lung injury. The results suggest that strategies to sequester extracellular vesicles could prevent their uptake and biologic cargo delivery to target cells, thereby reducing subsequent inflammatory injury; extracellular vesicle sequestration may therefore offer a therapeutic strategy in ARDS.

ARDS patients have a higher total concentration of alveolar extracellular vesicles, compared to control patients with hydrostatic edema [13]. A significant proportion of alveolar extracellular vesicles in ARDS patients are derived from alveolar epithelial cells; these extracellular vesicles contain higher concentrations of tissue factor and exert a pro-coagulant effect. Alveolar epithelial cell-derived extracellular vesicles may therefore also contribute to the increased pro-coagulant activity observed in ARDS and thereby represent a therapeutic target.

Different alveolar cell types release extracellular vesicles in sequential order following murine intratracheal LPS lung injury [14]. Alveolar macrophage-derived extracellular vesicles are rapidly released first, followed by endothelial extracellular vesicles and then neutrophil extracellular vesicles. This temporal difference in BALF extracellular vesicles release by different alveolar cell types may give insight into the pathological mechanisms underpinning ARDS. Alveolar macrophage-derived extracellular vesicle release may subsequently trigger pro-inflammatory extracellular vesicle release by epithelial cells and neutrophils. The alveolar macrophage extracellular vesicles can deliver high concentrations of TNF- α cargo to alveolar epithelial cells, resulting in increased production of the neutrophil chemotactic factor keratinocyte-derived cytokine (KC) and expression of intercellular adhesion molecule-1 (ICAM-1). BALF extracellular vesicles generated from intratracheal LPS treated mice resulted in lung injury when they were administered intratracheally to naïve mice, with increased alveolar neutrophil infiltration, alveolar protein permeability, and elevated BALF KC levels. Administration of these pathogenic BALF extracellular vesicles caused lung injury similar to LPS treatment. Alveolar macrophage extracellular vesicles containing TNF- α may play a significant role in instigating the inflammatory cascade in early ARDS; therefore alveolar macrophage extracellular vesicles should be considered as potential novel biomarkers and/or therapeutic targets.

Different modalities of lung injury can induce release of extracellular vesicles from different cell types. One group found that following sterile lung injury, most BALF extracellular vesicles were derived from type 1 alveolar epithelial cells [15]. However, following infectious lung injury, most BALF extracellular vesicles were derived from alveolar macrophages. BALF extracellular vesicles generated from

both sterile and infectious lung injury models promoted the recruitment of macrophages to the alveolar space. Sterile lung injury BALF extracellular vesicles (predominantly alveolar epithelial cell-derived) upregulated Toll-like receptor (TLR)2 and downregulated TLR8 expression in macrophages. Infectious lung injury BALF extracellular vesicles (predominantly alveolar macrophage-derived) upregulated TLR6 on macrophages. Differential effects on cytokine release were also observed with BALF extracellular vesicles: sterile injury extracellular vesicles upregulated alveolar macrophage release of IL-6 and TNF- α , whereas infectious injury extracellular vesicles upregulated IL-1 β and IL-10 release by alveolar macrophages. BALF extracellular vesicles generated following different modalities of lung injury promote inflammation via different pathways.

Remarkably, mouse models have indicated that release of extracellular vesicles following distant injury, e.g., traumatic brain injury [16] or trauma and hemorrhagic shock [17], can mediate lung injury. Following trauma and hemorrhagic shock, gut-derived extracellular vesicles were released into the mesenteric lymphatic system. Intravenous administration of these extracellular vesicles to naïve mice caused lung injury via macrophage TLR4 activation, including increased alveolar vascular permeability and inflammatory cell infiltration [17]. These findings indicate that similar mechanisms may be present in patients who develop ARDS following similar distant (indirect) insults. This pathway might explain in part the development of neurogenic pulmonary edema as well as lung injury following shock and ischemia-reperfusion.

Following lung injury, pro-inflammatory miRNAs can be transported between cells by extracellular vesicles. One group found that miR-17-5p was upregulated in BALF extracellular vesicles from patients with influenza A-induced ARDS; these extracellular vesicles were likely alveolar epithelial cell-derived. When influenza A infected alveolar epithelial cells were transfected with miR-17-5p, this downregulated expression of the antiviral factor MX1 and increased viral replication [18]. Other investigators found that BALF extracellular vesicles contained high concentrations of miR-466 in intratracheal-LPS mice [19]. Transfection of macrophages with miR-466 was found to upregulate the nucleotide-binding oligomerization like receptor 3 (NLRP3) inflammasome, and stimulate increased release of IL-1 β following LPS stimulation.

By learning how endogenous extracellular vesicles mediate inflammation and increase endothelial and epithelial permeability, it will be possible to gain greater insight into the protein and RNA pathways involved in the pathogenesis of ARDS. These pathogenic extracellular vesicles can be utilized as diagnostic/prognostic biomarkers, or as targets for novel therapeutic strategies.

4.3 Potential Endogenous Protective Effects of Extracellular Vesicles in ARDS

In the prior section, we discussed the evidence for how extracellular vesicles may contribute to ARDS pathogenesis; these harmful extracellular vesicles are predominantly derived from specific cell types (endothelium, alveolar epithelial cells, and alveolar macrophages). There is also evidence to suggest that extracellular vesicles

from other cell types may have a protective, anti-inflammatory role in the context of ARDS. Clinical studies investigating harmful and protective extracellular vesicles in ARDS patients are summarized in Table 4.1.

The data suggest that extracellular vesicles within a given biofluid cannot be considered as a homogenous entity; the origin and cargo of extracellular vesicles from different cell types at different stages of ARDS are likely to have divergent effects. Heterogeneity in cellular function and transcriptome has been shown to impact on patient outcomes in sepsis-related ARDS [20]. It is therefore likely that heterogeneity in extracellular vesicle profiles will similarly impact on patient outcome.

Table 4.1 Clinical studies investigating protective and pathological extracellular vesicles in patients with acute respiratory syndrome (ARDS)

Author [Ref.]	Cell origin	Biofluid	Patient cohort	Extracellular vesicle effect
<i>Pathological extracellular vesicles</i>				
Bastarache et al. [13]	Epithelial	BALF	11 ARDS patients and 13 hydrostatic edema patients	Epithelial extracellular vesicles are present at higher concentrations in ARDS patient BALF. Epithelial extracellular vesicles are enriched for tissue factor and likely contribute to increased pro-coagulant activity
Sun et al. [9]	Endothelial	Plasma	23 sepsis-related ARDS, 24 sepsis without ARDS and 19 non-sepsis patients	Elevated concentrations of endothelial extracellular vesicles containing S1PR3 are associated with increased mortality in ARDS patients
Scheller et al. [18]	Epithelial	BALF	6 Influenza A H1N1-induced ARDS patients	Influenza A induced ARDS patients have elevated concentrations of epithelial extracellular vesicles containing miR-17-5p. This miR increases viral replication within alveolar epithelial cells
<i>Protective extracellular vesicles</i>				
Guervilly et al. [21]	Leukocyte Neutrophil Endothelial Platelet	BALF Plasma	52 ARDS patients, 10 non-ARDS ventilated ICU patients and 12 spontaneously breathing patients	Leukocyte and neutrophil extracellular vesicle concentrations were elevated in ARDS patient BALF. In early ARDS, elevated BALF and plasma concentrations of leukocyte extracellular vesicles were associated with increased survival and VFDs
Neudecker et al. [26]	Neutrophil	BALF	55 ARDS patients	Neutrophil extracellular vesicles transfer miR-223 to alveolar epithelial cells, reducing permeability and inflammatory cytokine release
Shaver et al. [22]	All	Plasma	280 ICU patients at risk of ARDS. Of these 90 developed ARDS	Elevated plasma extracellular vesicle concentrations on ICU admission were associated with a lower risk of developing ARDS

BALF bronchoalveolar lavage fluid, *ICU* intensive care unit, *miR* micro ribonucleic acid, *S1PR3* sphingosine-1-phosphate receptor-3, *VFDs* ventilator-free days

Some clinical studies have reported that total leukocyte extracellular vesicle numbers are associated with a better prognosis in ARDS patients. An observational study characterized BALF and circulating extracellular vesicles from 52 ventilated patients with ARDS; control groups included ventilated patients without ARDS, and non-ventilated patients undergoing outpatient bronchoscopy [21]. The majority (90%) of ARDS patients had direct lung injury; 73% had pneumonia. The BALF from ARDS patients contained elevated leukocyte- and neutrophil-derived extracellular vesicles compared to controls. In early ARDS, elevated BALF and plasma concentrations of leukocyte extracellular vesicles were associated with increased survival and ventilator-free days, thus suggesting a potential role for BALF and serum leukocyte extracellular vesicles as prognostic biomarkers in early ARDS. In a separate study, total plasma extracellular vesicle concentrations were measured in 280 critically ill patients on intensive care unit (ICU) admission; 90 of these patients subsequently developed ARDS [22]. Elevated plasma extracellular vesicle concentrations were associated with a lower risk of developing ARDS; this association was seen most strongly in patients admitted to ICU with sepsis. Another group of investigators [23] found that a subset of circulating leukocyte extracellular vesicles expressing $\alpha 2$ -macroglobulin (A2MG) were associated with survival in ICU patients with sepsis secondary to pneumonia, but not in patients with sepsis secondary to fecal peritonitis. A2MG-EV treatment *in vitro* reduced endothelial cell permeability and increased bacterial phagocytosis by neutrophils.

Several studies have revealed a protective role for neutrophil extracellular vesicles in lung injury. Binding of neutrophil extracellular vesicles to Mer tyrosine kinase (MerTK) receptors on macrophages increased secretion of the pro-repair factor TGF β and decreased secretion of pro-inflammatory cytokines TNF- α and IL-8 [24, 25]. Therefore, neutrophil extracellular vesicles have an anti-inflammatory effect on macrophages. Neutrophil extracellular vesicles containing miR-223 were found to have an anti-inflammatory effect on alveolar epithelial cells, via suppression of poly(adenosine diphosphate-ribose) polymerase-1 [26]. In murine staphylococcal or ventilator-induced lung injury (VILI), intratracheal delivery of extracellular vesicles containing miR-223 reduces inflammatory cytokine release, alveolar protein permeability, and lung injury. Infiltration of neutrophils into the alveolar space is a hallmark of ARDS pathogenesis, and their pro-inflammatory role is established [27]. However, this evidence suggests that neutrophils may also have a concurrent anti-inflammatory role, via release of extracellular vesicles that modulate alveolar macrophage and alveolar epithelial cell functions.

Innate mechanisms that inhibit release or promote clearance of pro-inflammatory extracellular vesicles may be present in ARDS patients. Alveolar macrophages can phagocytose inflammatory BALF extracellular vesicles via MerTK binding, to prevent their uptake by alveolar epithelial cells [28]. The inflammatory BALF extracellular vesicles have a more injurious effect on alveolar epithelial cells compared to alveolar macrophages. As discussed in the previous section, LPS-stimulated macrophages release extracellular vesicles containing TNF- α , which can initiate an inflammatory cascade. LPS-stimulated alveolar epithelial cells can release IL-25, which acts on macrophages to suppress release of

inflammatory extracellular vesicles containing TNF- α [29]. Strategies to enhance IL-25 signaling or alveolar macrophage phagocytosis of extracellular vesicles may have therapeutic benefit in ARDS.

Subsets of leukocyte-derived extracellular vesicles appear to have a protective role in ARDS, which may be related to delivery of anti-inflammatory miRNAs. Mechanisms also exist to either inhibit inflammatory extracellular vesicle release or prevent their uptake by susceptible cell types. Therapeutic strategies to upregulate innate protective extracellular vesicles or enhance existing protective mechanisms may attenuate inflammation in ARDS.

4.4 Benefits of Extracellular Vesicles Derived from Mesenchymal Stromal Cells and Endothelial Progenitor Cells in ARDS Models

Extracellular vesicles derived from non-pulmonary cell types can have a protective effect in some models of ARDS. Mesenchymal stromal cells (MSCs) can attenuate inflammation and lung injury in preclinical models of ARDS, due to their intrinsic anti-inflammatory abilities [30]. Mesenchymal stromal cells mediate their effects via cell-cell contact and via release of paracrine factors; administration of mesenchymal stromal cell conditioned media was previously shown to attenuate lung injury in intratracheal-LPS injured mice [31]. Mesenchymal stromal cell-derived extracellular vesicles isolated from conditioned media attenuated inflammation and lung injury in both intratracheal-LPS and *E. coli* pneumonia models of murine lung injury [32, 33]. Prophylactic treatment with mesenchymal stromal cell extracellular vesicles increased survival in rats undergoing traumatic lung injury; inflammatory cytokines, infiltrating leukocytes, and pulmonary edema were all reduced [34]. These effects were in part due to mesenchymal stromal cell extracellular vesicle transfer of miR-124 [34]. In a pig model of influenza-induced lung injury, administration of mesenchymal stromal cell extracellular vesicles similarly reduced lung injury, alveolar protein permeability, and inflammatory cytokine release. Mesenchymal stromal cell extracellular vesicle treatment of alveolar epithelial cells reduced viral replication and virus-induced apoptosis. In addition, an experimental model of infant respiratory distress syndrome and bronchopulmonary dysplasia in newborn mice showed a therapeutic effect of extracellular vesicles isolated from mesenchymal stromal cells in reducing lung injury and restoring lung function, in part through induction of anti-inflammatory and pro-resolving macrophages [35].

Mesenchymal stromal cell extracellular vesicles can transfer mitochondria to alveolar macrophages, inducing a modified M2 (pro-resolving) phenotype [36]. Mesenchymal stromal cells stimulated with IL-1 β release extracellular vesicles containing miR-146a, which also induces an M2 macrophage phenotype [37]. These mesenchymal stromal cell extracellular vesicle modified alveolar macrophages have pro-resolving characteristics (increased secretion of anti-inflammatory cytokine IL-10, reduced secretion of inflammatory cytokines TNF- α and IL-8), but also

increased phagocytic activity against bacteria [31, 36, 38, 39]. Mesenchymal stromal cell extracellular vesicles modulate alveolar macrophages to clear bacteria more effectively, while minimizing surrounding tissue injury.

Mesenchymal stromal cell extracellular vesicles also contain mRNA for keratinocyte growth factor (KGF) and angiopoietin-1, which can be transferred to alveolar epithelial cells and endothelial cells [32, 40], thereby increasing the integrity of the alveolar-capillary barrier. These findings explain the ability of mesenchymal stromal cell extracellular vesicles to restore alveolar fluid clearance in *ex vivo* human lungs [41]. In an *E. coli* pneumonia model using *ex vivo* human lungs, administration of mesenchymal stromal cell extracellular vesicles reduced bacterial load within the alveolar space, reduced protein permeability, and increased alveolar fluid clearance [42]. Mouse studies indicated that mesenchymal stromal cell extracellular vesicle transfer of miR-145 to macrophages was responsible for the increased bacterial phagocytosis [43].

Endothelial progenitor cells (EPCs) also release extracellular vesicles that have a protective role in lung injury. Endothelial progenitor cells release extracellular vesicles containing miR-126, which are taken up by endothelial cells [44], resulting in enhanced endothelial cell proliferation, migration, angiogenesis, and trans-epithelial electrical resistance. Administration of endothelial progenitor cell extracellular vesicles decreased lung injury, hypoxia, alveolar cell count, protein permeability, pulmonary edema, and inflammatory cytokines in the murine intratracheal-LPS model [44, 45]. Work in human small airway epithelial cells found that miR-126 could increase expression of tight junction proteins [45]. Therefore, endothelial progenitor cell extracellular vesicles containing miR-126 have a protective effect on both the epithelium and endothelium in models of ARDS.

Mesenchymal stromal cells and endothelial progenitor cells mediate their anti-inflammatory and pro-repair effects in part by release of extracellular vesicles, which deliver miRNA, mRNA, and mitochondrial cargo to different alveolar cell types. Administration of mesenchymal stromal cell extracellular vesicles or endothelial progenitor cell extracellular vesicles may therefore offer a novel therapeutic strategy for ARDS patients.

4.5 Clinical Applications for Extracellular Vesicles in ARDS

As summarized in Fig. 4.1, extracellular vesicles have wide-ranging potential clinical applications in ARDS. Extracellular vesicles from specific cell types could be used as diagnostic or prognostic biomarkers. Human endothelial extracellular vesicles can induce lung injury in mice [6], and a subset of endothelial extracellular vesicles has been associated with mortality in ARDS patients [9]. Therefore, endothelial extracellular vesicles could be used as diagnostic and prognostic biomarkers in ARDS. By understanding how extracellular vesicles mediate intercellular transfer of genetic material, organelles, and proteins between different cell types in the alveolar space, it will be possible to learn how the RNA and protein pathways of injury are involved in ARDS pathogenesis.

Circulating and BALF pathogenic extracellular vesicles offer therapeutic targets in ARDS. Studies have thus far identified endothelial- and alveolar macrophage-derived extracellular vesicles as having the ability to induce lung injury [6, 14]. Therapeutic targeting of pathogenic extracellular vesicles could prevent transfer of pro-inflammatory genetic material and proteins to target cells. Nonspecific extracellular vesicle sequestering with hyaluronic acid [46] would target all extracellular vesicles expressing CD44 (a widely expressed glycoprotein), but has a potential disadvantage of sequestering both pathogenic and beneficial extracellular vesicles.

Alveolar macrophages can phagocytose pathogenic extracellular vesicles via MerTK receptors, thereby preventing extracellular vesicle uptake by alveolar epithelial cells and subsequent inflammatory injury [28]. Therapeutic strategies to upregulate MerTK expression on alveolar macrophages may aid uptake of pathogenic extracellular vesicles and attenuate inflammation in ARDS. Strategies to inhibit pathogenic extracellular vesicle release also require consideration: alveolar epithelial cells can release IL-25 to inhibit the release of pathogenic alveolar macrophage extracellular vesicles [29]. Simvastatin can inhibit the release of pathogenic endothelial extracellular vesicles in murine models of lung injury [10] and this may be relevant in the setting of ARDS [11]. Surprisingly, neutrophil extracellular vesicles have been shown to have an anti-inflammatory role in sepsis and ARDS [25, 26]. Therefore, strategies to stimulate extracellular vesicle release by neutrophils *in vivo* or administration of neutrophil extracellular vesicles generated *ex vivo* could be considered.

Extracellular vesicles derived from exogenous mesenchymal stromal cells and endothelial progenitor cells mediate the anti-inflammatory actions of the parent cell by delivery of mitochondria, genetic material, and proteins to injured alveolar cells. As described above, therapeutic use of mesenchymal stromal cell extracellular vesicles has shown efficacy at reducing lung injury in several preclinical models. In mouse models of lung injury, mesenchymal stromal cell extracellular vesicles have similar efficacy to mesenchymal stromal cells themselves. Finally, exogenous extracellular vesicles could be modified to package custom drugs or protective RNA cargo, which could be delivered to specific cell types as determined by the extracellular vesicle surface markers [47].

4.6 Future Directions

Several studies that have investigated the role of extracellular vesicles in acute lung injury and ARDS have been done in animal and *in vitro* models, although one recent study was done in the *ex vivo* perfused human lung [12], and there are a few clinical studies as well [13, 18, 21–23]. Future studies will need to characterize BALF and circulating extracellular vesicles from ARDS patients with regard to the cell of origin, cargo assessment (RNA, protein, organelle content), and their biological effect on human cells and human tissues. Standardized methods of biologic fluid collections and RNA isolation from extracellular vesicles are now possible, so that the data will be comparable and generalizable [48]. Extracellular vesicle profiles from ARDS patients will need to be compared with those from animal models and *ex vivo*

human lung models in order to determine how well the extracellular vesicles released in these models correlate with those observed in the clinical setting of ARDS. Clinical studies to test the utility of extracellular vesicles as diagnostic and prognostic biomarkers in ARDS patients will be needed. Clinical trials of mesenchymal stromal cell extracellular vesicles are needed to determine therapeutic utility in patients with ARDS as well as infant respiratory distress syndrome and bronchopulmonary dysplasia.

4.7 Conclusions

Extracellular vesicles constitute an important intercellular communication mechanism, which allows targeted transfer of biologic cargo including RNA, micro RNA, proteins, and mitochondria between different cell types. New evidence indicates that extracellular vesicles are likely to be critical to the induction and resolution of injury in ARDS. Consequently, it is likely that there will be a wide range of clinical applications for extracellular vesicles, ranging from use as biomarkers to therapeutic agents for ARDS.

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