Myeloid Extracellular Vesicles

Mahida, Rahul Y; Matthay, Michael A

DOI:
10.1165/rcmb.2022-0380ED

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (Harvard):
Mahida, RY & Matthay, MA 2023, 'Myeloid Extracellular Vesicles: New Players in Indirect Lung Injury', *American Journal of Respiratory Cell and Molecular Biology*, vol. 68, no. 2, pp. 121-123.
https://doi.org/10.1165/rcmb.2022-0380ED

Myeloid Extracellular Vesicles: New Players in Indirect Lung Injury

Infection, either direct (pulmonary) or indirect (extrapulmonary), is the most common precipitating factor for the development of acute respiratory distress syndrome (ARDS). Since the coronavirus disease (COVID-19) pandemic, we have started to develop pharmacotherapies specifically for patients with ARDS; however, our knowledge of the underlying pathogenic mechanisms remains incomplete. Determining the pathways that lead from sepsis to ARDS will allow identification of novel therapeutic strategies. Extracellular vesicles (EVs) are membrane-bound anuclear structures that constitute an intercellular communication mechanism, allowing transfer of biologic cargo (including microRNA, mRNA, proteins, and mitochondria) between cell types (1). Cells release EVs during both health and disease states. EVs have been shown to play a pathogenic (and at times protective) role across various inflammatory diseases and cancers, in which EVs act as biomarkers and/or potential therapeutic targets (2). There is an increasing body of evidence to support the role of EVs and their cargo in the pathogenesis of acute lung injury (ALI) and ARDS. EVs are released when human *ex vivo* perfused lungs are injured with *Escherichia coli*; these EVs then mediate inflammatory lung injury when isolated and administered to the perfusate of uninjured human lungs (3). Cellular infiltration, elevated inflammatory cytokine release, and reduced alveolar fluid clearance are observed.

In this issue of the *Journal* (pp. 140–149), Tan and colleagues (4) describe their investigation of the role of EVs in mediating indirect ALI; *in vivo*–generated circulating EVs were extracted from LPS-treated mice and administered to the perfusate of *ex vivo* isolated perfused lungs (IPL). The authors found that circulating LPS-induced total EVs can induce indirect ALI, as characterized by pulmonary edema measured as an increase in the lung wet/dry ratio and elevated concentrations of a biomarker of alveolar epithelial type 1 cell injury, RAGE (receptor for advanced glycation end products), in the IPL model. This EV-mediated lung injury is dependent on the presence of pulmonary intravascular monocytes, as depletion of monocytes via use of clodronate liposomes in the IPL model nullified EV-mediated injury. The major strength of this study is that the authors used an innovative murine *in vivo*–to–*ex vivo* adoptive transfer approach to demonstrate the role of EVs in mediating indirect ALI. The authors subsequently found that the myeloid-derived cluster of differentiation molecule ($CD11b^{+}$) subpopulation of EVs predominantly mediates this injurious effect. Indirect lung injury commences with inflammatory injury to the pulmonary endothelium. The authors subsequently undertook *in vitro* studies showing that human myeloid-derived EVs induce inflammatory injury in a human lung microvascular endothelial cell–peripheral blood mononuclear cell coculture, with increased cell adhesion molecule and inflammatory cytokine expression. The injurious effect of myeloid-derived EVs was dependent on the presence of peripheral blood mononuclear cells. Together, these studies provide convincing evidence that in indirect ALI, the injurious effect of myeloid-derived EVs on the pulmonary vasculature is monocyte dependent (Figure 1). Granulocyte-derived EVs have previously been shown to have a proinflammatory effect on monocytes *in vitro* (5). Previous murine studies have similarly shown that depletion of alveolar macrophages via clodronate liposomes attenuated both ventilator- and LPS-induced lung injury (6–8).

The profile of LPS-induced circulating EVs differs between murine and human studies. Murine *in vivo* models showed an increase in both neutrophil- and monocyte-derived EVs after LPS stimulation. However, healthy volunteer whole blood showed an increase in neutrophil-derived, but not monocyte-derived, EVs after *in vitro* LPS stimulation, which indicates a limitation of this model. A recent analysis of pulmonary EVs revealed that both monocyte- and neutrophil-derived EVs are elevated in patients with sepsis (9). Comparison of patients with sepsis, with and without ARDS, revealed that monocyte-derived EVs were elevated in patients with ARDS; however, the majority of these patients had direct lung injury. Circulating monocyte-derived EVs from patients with sepsis-related ARDS have been found to contain caspase-1 and gasdermin D; after uptake, these EVs induce pulmonary endothelial cell death (10). Thus, the role of monocyte-derived EVs within the myeloid EV population should also be considered.

The authors address previous work, which partly contradicts their findings, showing that activated neutrophil-derived EVs have an antiinflammatory effect on macrophages (11, 12). Neutrophil-derived EVs from patients with ARDS and murine models of ALI also have a protective effect on airway epithelial cells; transfer of microRNA-223 (miR-223) cargo downregulates PARP-1 (poly[adenosine diphosphate–ribose] polymerase–1) expression, reducing permeability and inflammatory cytokine release. The authors hypothesize that neutrophil-derived EVs may have a differential impact on monocytes (inflammatory) versus macrophages (antiinflammatory). This differential response may be due to the increased expression of integrin and scavenger receptors on the surface of macrophages that bind EVs. It has previously been shown that during inflammatory conditions, airway epithelial cells take up more EVs and undergo activation, whereas in contrast alveolar macrophages take up fewer EVs and show no change in activation (13); a similar dichotomy may occur with monocytes and macrophages. Studies directly comparing the biological effect of neutrophil-derived EV uptake by monocytes and macrophages are required to confirm this. Further studies to characterize neutrophil-derived EV cargo will also help extend our understanding of their effect on target cells and, consequently, the initiation and resolution of inflammation in ARDS.

Previous *in vitro* and *in vivo* models of sepsis-associated ARDS have recently reported that EVs containing miR-146a-5p activate TLR-7 (Toll-like Receptor 7) in macrophages, resulting in inflammatory injury to the pulmonary endothelium with increased

Originally Published in Press as DOI: 10.1165/rcmb.2022-0380ED on October 10, 2022

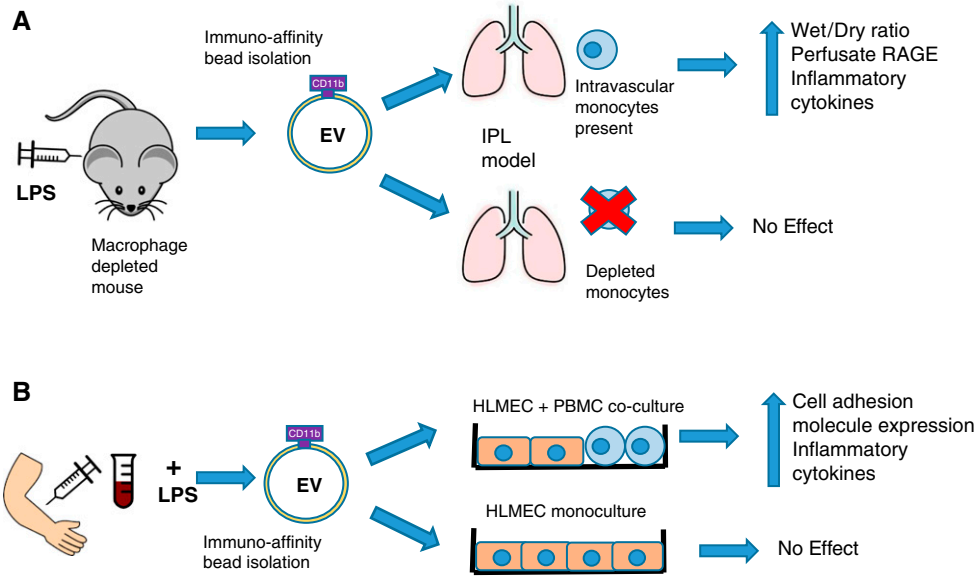

Figure 1. Myeloid extracellular vesicle (EV)–mediated endothelial injury is dependent on monocytes. (*A*) Mice were depleted of intravascular macrophages before treatment with intravenous LPS. Myeloid-derived ($CD11b^{+}$) EVs were isolated from the circulation and added to the perfusate of murine *ex vivo* isolated perfused lungs (IPLs). In the presence of intravascular monocytes, myeloid EVs induced lung injury. However, if the IPLs were depleted of intravascular monocytes, administration of myeloid EVs had no effect. (*B*) Whole blood taken from healthy volunteers was stimulated with LPS, and myeloid-derived EVs were isolated. Myeloid EVs were added to either human lung microvascular endothelial cell (HLMEC)–peripheral blood mononuclear cell (PBMC) cocultures or HLMEC monocultures; endothelial injury was only observed in the presence of PBMCs. CD11b = cluster of differentiation molecule; RAGE = receptor for advanced glycation end products.

endothelial permeability (14, 15). However, in the absence of macrophages, there was no endothelial injury. These findings support those of Tan and colleagues (4), indicating that monocytes and macrophages may play a key role in mediating EV-induced endothelial injury in sepsis-related ARDS. It would be of great interest to learn whether the myeloid-derived EVs isolated in this current study were enriched in miR-146a-5p, which would in part reveal how these EVs mediate their biological effects. A limitation of this study is that the authors did not analyze the cargo of myeloid-derived EVs. A single EV may often contain multiple different genetic, protein, and organelle cargoes. Thus, characterization of EV cargo, including the proteome, microRNA and mRNA transcriptome, is an important part of determining the biological effects of EV subtypes on target cells (e.g., intravascular monocytes) and their role in ARDS pathogenesis. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Rahul Y. Mahida, M.B. B.S., Ph.D.
Institute of Inflammation and Ageing
University of Birmingham
Birmingham, United Kingdom

Michael A. Matthay, M.D.
Cardiovascular Research Institute
University of California San Francisco
San Francisco, California

ORCID ID: 0000-0003-1100-8684 (R.Y.M.).

References

1. Mahida RY, Matsumoto S, Matthay MA. Extracellular vesicles: a new frontier for research in acute respiratory distress syndrome. *Am J Respir Cell Mol Biol* 2020;63:15–24.
2. Wang X, Tian L, Lu J, Ng IO. Exosomes and cancer: diagnostic and prognostic biomarkers and therapeutic vehicle. *Oncogenesis* 2022; 11:54.
3. Liu A, Park JH, Zhang X, Sugita S, Naito Y, Lee JH, *et al.* Therapeutic effects of hyaluronic acid in bacterial pneumonia in *ex vivo* perfused human lungs. *Am J Respir Crit Care Med* 2019;200: 1234–1245.
4. Tan YY, O'Dea KP, Tsiridou DM, Pac Soo A, Koh MW, Beckett F, *et al.* Circulating myeloid cell-derived extracellular vesicles as mediators of indirect acute lung injury. *Am J Respir Cell Mol Biol* 2023;68:140–149.
5. Danesh A, Inglis HC, Abdel-Mohsen M, Deng X, Adelman A, Schechtman KB, *et al.* Granulocyte-derived extracellular vesicles activate monocytes and are associated with mortality in intensive care unit patients. *Front Immunol* 2018;9:956.
6. Eyal FG, Hamm CR, Parker JC. Reduction in alveolar macrophages attenuates acute ventilator induced lung injury in rats. *Intensive Care Med* 2007;33:1212–1218.
7. Frank JA, Wray CM, McAuley DF, Schwendener R, Matthay MA. Alveolar macrophages contribute to alveolar barrier dysfunction in ventilator-induced lung injury. *Am J Physiol Lung Cell Mol Physiol* 2006;291:L1191–L1198.
8. Koay MA, Gao X, Washington MK, Parman KS, Sadikot RT, Blackwell TS, *et al.* Macrophages are necessary for maximal nuclear factor-kappa B activation in response to endotoxin. *Am J Respir Cell Mol Biol* 2002;26: 572–578.
9. Mahida RY, Price J, Lugg ST, Li H, Parekh D, Scott A, *et al.* CD14-positive extracellular vesicles in bronchoalveolar lavage fluid as a new biomarker of acute respiratory distress syndrome. *Am J Physiol Lung Cell Mol Physiol* 2022;322: L617–L624.

10. Mitra S, Exline M, Habyarimana F, Gavrilin MA, Baker PJ, Masters SL, *et al.* Microparticulate caspase 1 regulates gasdermin D and pulmonary vascular endothelial cell injury. *Am J Respir Cell Mol Biol* 2018;59:56–64.
11. Eken C, Martin PJ, Sadallah S, Treves S, Schaller M, Schifferli JA. Ectosomes released by polymorphonuclear neutrophils induce a MerTK-dependent anti-inflammatory pathway in macrophages. *J Biol Chem* 2010;285:39914–39921.
12. Gasser O, Schifferli JA. Activated polymorphonuclear neutrophils disseminate anti-inflammatory microparticles by ectocytosis. *Blood* 2004;104:2543–2548.
13. Soni S, O'Dea KP, Abe E, Khamdan M, Shah SV, Sarathchandra P, *et al.* Microvesicle-mediated communication within the alveolar space: mechanisms of uptake by epithelial cells and alveolar macrophages. *Front Immunol* 2022;13:853769.
14. Huang H, Zhu J, Gu L, Hu J, Feng X, Huang W, *et al.* TLR7 mediates acute respiratory distress syndrome in sepsis by sensing extracellular miR-146a. *Am J Respir Cell Mol Biol* 2022;67:375–388.
15. Xu J, Feng Y, Jeyaram A, Jay SM, Zou L, Chao W. Circulating plasma extracellular vesicles from septic mice induce inflammation via microRNA- and TLR7-dependent mechanisms. *J Immunol* 2018;201: 3392–3400.