Macrophage-derived microvesicles' pathogenic role in acute lung injury

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Once considered cellular debris or, more recently, as biomarkers of disease progression, extracellular vesicles (EVs), comprised of exosomes, microvesicles (MVs) and apoptotic bodies, released by living and dying eukaryotic cells are now recognised as important mediators of cellular communication and function.[1] EVs are a heterogeneous population of anucleate vesicles with a diameter of 100–1000 nm that are released from intracellular compartments as exosomes or by budding off the plasma membrane as MVs in response to diverse physiological, pathophysiological or external stimulus. Despite its importance in various organ injuries and cancer,[2 3] the role of EVs in the pathogenesis of acute lung injury (ALI) is still largely unexplored.[4–6] In the current issue of *Thorax*, Soni *et al*[7] studied the temporal pattern of EV release in early ALI and found that MVs from alveolar macrophages were the predominant source and played a significant role as potent initiators of the inflammatory response in part by the transfer of its cargo, the cytokine tumour necrosis factor (TNF), to target cells.

By inducing ALI with intratracheal instillation of lipopolysaccharide (LPS) in C57BL6 mice, the authors found that MVs released into the injured alveolus were mainly from alveolar macrophages initially by flow cytometry, peaking at 1 hour and followed soon thereafter by MVs released by alveolar epithelial cells and neutrophils by 4 hours. More importantly, the MVs in the bronchoalveolar fluid (BALF) at 1 hour contained a substantial amount of TNF but low levels of interleukin (IL)-1β and IL-6 and induced a mouse lung epithelial (MLE)-12 cell line to express intercellular adhesion molecule 1 (ICAM-1) and secrete KC, a murine IL-8 homologue, at 4 hours. Surprisingly, alveolar LPS-generated MVs were almost twice more potent than a dose of LPS (100 μg/mL) used as a positive control group on the expression of ICAM-1 and secretion of KC by MLE-2 cells (Figure 6). In experiments to study the mechanisms of MVs, the authors found that MVs released by primary cultures of mouse macrophages primed with LPS to obtain a pro-inflammatory phenotype also induced ICAM-1 expression in MLE-12 cells, which was dependent on the TNF cargo of the MVs. When instilled intratracheally in naive mice, MVs isolated from macrophages primed with LPS induced ALI with an increase in influx of neutrophils and cytokines/chemokines (eg, KC), in lung protein permeability and in the expression of ICAM-1 on alveolar epithelial type I and II cells (Figure 8).[7]

There are several important findings in the manuscript by Soni *et al*[7] with clinical relevance. First, the clear demonstration that MVs released into the injured alveolus early in ALI were biologically active, with the capability to induce an inflammatory response. All experiments had appropriate controls such as MVs isolated from the BALF from mice without injury or the supernatant from the washing steps during the isolation of the MVs as well as low and high doses of LPS to ensure the inflammatory response was specific to the MVs, not contaminants. Second, in early ALI following intratracheal LPS instillation, alveolar macrophages were the initial source of the pro-inflammatory MVs, followed soon thereafter by MVs released from alveolar epithelial cells and neutrophils, as determined by flow cytometry. And finally, the protein cargo, for example, TNF, was one of the mechanisms through which MVs induced an inflammatory response.

There are limitations to the manuscript that require further study to more clearly define the role of EVs released during ALI. In the context of early ALI, what are the roles of exosomes or apoptotic bodies in the inflammatory response? What about other cellular sources such as platelets, which are a major source of EVs and play a critical role in ALI?[8] The phenotype of the cells may determine the functional effects of the released MVs. For example, several studies have shown a key role of alveolar macrophages with an anti-inflammatory M2 phenotype in the resolution of ALI.[9] The functional effect of the released MV from alveolar macrophages may be both pro-inflammatory and anti-inflammatory, depending on when the MVs were isolated during the course of ALI. And lastly, although the protein cargoes of MVs are important, the role of mRNA and microRNA in the MVs should not be overlooked. Multiple studies have shown a transfer of the RNA content between MVs and cells with subsequent translation of the mRNA or microRNA leading to a functional effect.[2 3 10] In the current study, the inflammatory response following MVs' administration in vivo or in vitro may be artificially diminished due to the short incubation time (4 hours), which may not be enough for the translation of the RNA cargo. One wonders, if the incubation time was increased, whether the inflammatory response would be greater.

As with any manuscript with findings with potential translational impact, more questions are raised than answered. How critical are MVs, exosomes and apoptotic bodies in the pathophysiology of ALI compared with the released soluble factors, for example, cytokines and chemokines, especially in terms of the early inflammatory phase? Soni *et al*[7] found minimal levels of IL-1β and IL-6, inflammatory cytokines with significant inflammatory roles in ALI, in the MVs derived predominantly from alveolar macrophages. However, Eltom *et al* and others have shown that increased airway ATP levels, commonly found in patients with respiratory diseases such as asthma and COPD, can stimulate the release of IL-1β/IL-18 from EVs released during infection.[11] Can MVs, exosomes or apoptotic bodies be targeted and neutralised during the early inflammatory phase of ALI, possibly preventing the progression to acute respiratory distress syndrome? Will the molecular cargo of the released MVs, exosomes or apoptotic bodies differ based on the cause of ALI, for example, LPS versus bacterial pneumonia versus sepsis versus ventilator-induced lung injury? For example, are EVs released by endothelial cells potentially more critical in indirect ALI such as following abdominal sepsis for the initiation of the inflammatory response in the lung[12]? Although the focus of the current study was on MVs derived from alveolar macrophages, clearly MVs derived from neutrophils have significant roles during ALI. Timár *et al*[13] recently found that MVs released from primary cultures of human neutrophils had bacteriostatic properties. And as alluded to as a limitation, the number and phenotype of the EVs will markedly change prior to, during and in the resolution phase of ALI. Using surface membrane markers to identify the source of the MVs by flow cytometry may be

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inadequate in describing the biological role of these vesicles. Clearly, more studies are required and new techniques may need to be developed.

Despite extensive research and numerous preclinical studies identifying various biological mediators, there are no specific pharmacological therapies for acute respiratory distress syndrome, the clinical form of ALI, and treatment is largely limited to supportive care and lung-protective ventilation.[14–16] The manuscript by Soni *et al* adds to the understanding of the biological role of EVs in the pathophysiology of acute respiratory distress syndrome, which may ultimately yield new therapeutic targets and reinvigorate an area of study of ALI where the translation of preclinical success to clinical use has been modest.

Funding R01HL-113022 from the NIH National Heart, Lung, and Blood Institute (NHLBI) (USA).

Competing interests None declared.

Provenance and peer review Commissioned; externally peer reviewed.

To cite Lee JW. *Thorax* 2016;**71**:975–976.

Published Online First 18 August 2016

▶ http://dx.doi.org/10.1136/thoraxjnl-2015-208032

Thorax 2016;**71**:975–976.
doi:10.1136/thoraxjnl-2016-208895

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