ExRNA Takes a Toll in Sepsis-associated Lung Injury

Following cell stress, injury, or infections, potentially injurious host-derived damage-associated molecular patterns (DAMPs) trigger the innate immune response. Among these DAMPs, the importance of extracellular nucleic acids, such as mitochondrial DNA and vesicle-associated noncoding RNA, in the pathogenesis of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), is increasingly recognized. Extracellular RNA (exRNA) is primarily composed of microRNA (miRNA) and ribosomal RNA (rRNA) and can be vesicle-associated, protein-bound, or free form. These different forms of exRNA play roles in endothelial permeability and inflammation via recognition by receptors such as VEGFR-2 (vascular endothelial growth factor receptor 2) (1), receptor for advanced glycation end products (RAGE) (2), and toll-like receptors (TLRs). Plasma and alveolar exRNA profiles are altered during lung injury and sepsis (3), and plasma microRNA-146–5p (miR146–5p) is elevated during sepsis (4). Given the diverse sequences and forms of exRNA and increasing interest in RNA-based therapeutics, understanding how some of these exRNAs contribute to lung injury or other diseases will advance our technology in applying RNA biology to mitigate inflammatory diseases and clinical syndromes including sepsis and acute lung injury.

In this issue of the *Journal*, Huang and colleagues (pp. 375–388) report that extracellular miR146a-5p activates TLR7 in macrophages and induces vascular permeability via TNFα (5). miR146a-5p is a miRNA that negatively regulates TLR signaling intracellularly by silencing IRAK1/TRAF6 expression (6). However, the extracellular form of miR146a-5p is vesicle-associated and is proinflammatory in macrophages (7). Thus, miR146a-5p fulfills the definition of a DAMP. In a cecal ligation and puncture-induced model of sepsis, miR146a-5p is elevated in both plasma and BAL. Intra-tracheal administration of miR146a-5p induced TLR7-dependent proinflammatory cytokine expression, endothelial disruption, and neutrophil recruitment. *In vitro*, conditioned media from miR146a-5p-treated macrophages and septic mice increased permeability in a large vessel (HPAEC) and microvascular (HMVEC) lung endothelial cells. Direct treatment of endothelial cells, which lack TLR7 expression at rest, with miR146–5p did not affect endothelial integrity, suggesting the effects of miR146a-5p were indirect and due to a macrophage-derived mediator. In addition, conditioned media from macrophages exposed to noninflammatory miR210–3p or a mutant nonimmunostimulatory miR146a-5p did not disrupt endothelial integrity. Using a protein array, TNFα was found to be differentially induced by miR146a-5p via TLR7; concomitantly, neutralizing TNFα in the conditioned media and septic sera prevented vascular permeabilization. Overall, the results demonstrate that one mechanism for lung injury following peritoneal sepsis is macrophage TLR7-dependent TNFα production in response to extracellular miR146a-5p.

The major strength of this study is the use of mice to demonstrate that miR146a-5p is sufficient to induce lung inflammation, followed by a reductionist approach to identify the corresponding mechanism *in vitro*. This shows the relevance of miR146a-5p in the whole organism and identifies a potential target for intervention. Furthermore, *in vitro* macrophage stimulation with noninflammatory miR210–3p or mutant of miR146a-5p failed to disrupt endothelial integrity, indicating the specificity for TLR7-sensing of miR146a-5p. This study also used multiple methods to dissect endothelial disruption, revealing cytoskeletal and VE-cadherin modulation by TNFα. Although the immune response to miR146a-5p by bone marrow-derived macrophages may not directly represent the immune response to the complex composition of soluble mediators released during sepsis, the authors recapitulated their findings of TNFα-dependent endothelial disruption using plasma from septic mice in *ex vivo* studies. However, it remains to be determined whether peritoneal macrophages, which localize in the primary site of injury in the model, or alveolar macrophages, also contribute to lung inflammation induced by miR146a-5p. A limitation of this study is the paired use of murine macrophages or septic sera with human endothelial cells. Although the functions of these cell types are largely conserved, the results may not fully recapitulate the immune response in humans as the macrophage response to stimuli is not necessarily conserved (8, 9); this is further complicated by the added complexity during polymicrobial sepsis, where a multitude of DAMPs and PAMPs are present.

The finding that exRNA sensing by TLR7 resulted in organ injury has also been reported in other models (10, 11), including a model of liver injury-induced lung inflammation by miR122 and sepsis-induced brain injury by miR146a-5p, suggesting that TLR7 senses multiple miRNAs and that the miRNA-TLR7 pathway is a potential target for lung and other remote organ injuries during sepsis and lung injury secondary to diverse insults. However, because differential cytokine and inflammatory responses are triggered in response to a diverse repertoire of exRNA and vesicles released during injury (12), the identity and functions of these different vesicular components, including exRNA, will need further investigation. Moreover, despite the seemingly injurious role of TLR7 in these models, deficiency of TLR7 can exacerbate lung injury, as illustrated in patients with X-linked TLR7 deficiency and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (13). Thus, understanding the dynamics and kinetics of how TLR7 interacts with different RNA DAMPs generated during infection and following sterile injury can enhance our knowledge and perhaps lead to targeting tissue-specific TLRs for inflammatory syndromes.

This study also raises other important questions. First, although TNFα was identified as a primary driver for endothelial dysfunction, neutralizing TNFα only partially suppressed endothelial disruption by septic sera, suggesting the involvement of additional, yet to be elucidated, factors contributing to endothelial dysfunction in this model of polymicrobial sepsis-induced lung injury. Except for IL-6

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blockade for coronavirus disease (COVID-19)–associated lung injury, no other cytokine therapy (including TNF blockade) has improved mortality in sepsis and sepsis-associated lung injury (14). Identifying miR146a-5p as an inducer of TNFα in sepsis is intriguing as this biomarker may be useful for patient selection if anti-TNFα trials are revisited in sepsis. Second, although extracellular miR146a-5p triggers TLR7-dependent inflammation, it also negatively regulates TLR signaling by silencing IRAK1 and TRAF6 expression intracellularly, suggesting that the localization of miRNA is essential for its function. The current work by Huang and colleagues suggests that extracellular RNA DAMPs contribute to the pathogenesis of sepsis-induced endothelial dysfunction and consequent lung injury. Collectively, these findings add to the growing body of literature demonstrating the importance of nucleic acid DAMPs in initiating and perpetuating lung injury. Future research on identifying the composition of exRNAs in diverse inflammatory states (including sepsis and ARDS), biological interactions of these exRNAs with classical and nonclassical immune cells, and the regulation of exRNAs in the circulation may not only identify potential biomarkers but also elucidate novel mechanisms of tissue injury during sepsis, thus adding to our armamentarium of potentially targetable pathways for precision-based approaches to sepsis-associated lung injury. ■

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L.K. Metthew Lam, Ph.D.
Nilam S. Mangalmurti, M.D.
University of Pennsylvania Perelman School of Medicine
Philadelphia, Pennsylvania

ORCID ID: 0000-0002-9146-1608 (N.S.M.).

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