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Mind the Gap between the Endothelium and E3 Ubiquitin Ligase: TRIM21 Is a Viable Therapeutic Target in Sepsis-induced Endothelial Dysfunction

Acute lung injury (ALI) due to sepsis or mechanical ventilation is a refractory lung disease that is characterized by severe hypoxemia and unacceptably high morbidity and mortality (>35%) (1). The underpinning pathology associated with ALI is intimately related to a profound vascular leak and alveolar flooding, the defining features of this syndrome. The sustained presence of vascular leak in unremitting ALI increases the likelihood of multiorgan failure and death (2). Although we have made advances in understanding ALI-associated lung pathophysiology, there are currently no specific therapies to alleviate vascular leak. The vascular endothelium and alveolar epithelium are functionally complex tissues that form a selective semipermeable barrier to regulate the passage of macromolecules and fluid from the vascular compartment and lung interstitium. Specifically, lung microvascular endothelial cells play a critical role in maintaining the integrity of the vascular barrier, and dysfunction of this barrier is an important underlying pathology in the alveolar flooding observed in patients with sepsis (3, 4).

Maintenance of endothelial barrier integrity is a complex process that is regulated by opposing intracellular contractile forces and adhesive cell–cell and cell matrix tethering forces (5). Studies performed over the past three decades in animal models of sepsis and acute respiratory distress syndrome, and *in vitro* with endothelial cell cultures, have focused on mechanisms that regulate endothelial permeability responses to edematic agents such as thrombin, LPS, histamine, and bradykinin, and high oxygen tension (6). However, several biomolecules, such as sphingosine-1-phosphate, FTY720 and its analogs, hepatocyte growth factor, activated protein C, high-molecular-weight hyaluronan, and oxidized phospholipids, are known to act as barrier-enhancing agents that retard and reverse inflammatory lung edema *in vitro* and *in vivo* (7, 8). Endothelial cell permeability induced by barrier-disruptive agents involve signaling pathways mediated through mitogen-activated protein kinases, endothelial myosin light chain kinase, and Rho GTPases that regulate actomyosin-dependent disruption of cell junction and adherens junction proteins (5, 6). On the contrary, barrier-enhancing agents enhance the interaction between actin and cortactin, and α/β-catenin and VE-cadherin at adherens junctions and leading edges of cells, thereby promoting lamellipodia formation, wound healing, and cell migration (7, 9). However, detailed interactions between the barrier-disruptive and barrier-enhancing signaling pathways that regulate endothelial barrier integrity are poorly defined.

TRIM21 (tripartite motif 21, also known as Ro52) is an ~52 kD protein that is predominantly expressed in hematopoietic cells, as well as in endothelial and epithelial cells (10). TRIM21 is an E3 ubiquitin ligase that catalyzes the ubiquitination of proteins such as IFN-regulatory factor and several TRIM family members (11). Furthermore, TRIM21 is an effector molecule for intracellular antibodies, as well as an intracellular Fc receptor that links cytosolic antibody recognition to the ubiquitin proteosome system (12). It is also an autoantigen that is recognized by anti-TRIM21 autoantibodies in sera of patients with systemic lupus erythematosus and Sjogren's syndrome (10). Studies have shown that decreased expression of TRIM21 promotes cell growth in breast cancer cells (13), *Trim21* deficiency regulates NF-κB–dependent proinflammatory cytokine production in fibroblasts (14), and augmented T-helper cell type 17 differentiation in TRIM21 deficiency promotes a more fibrous, stable phenotype of atherosclerotic plaques with high collagen content (15). These studies demonstrated several intrinsic roles for TRIM21 in human pathologies such as breast cancer, inflammation, atherosclerotic plaque stabilization, and the innate immune response to viral-RNA infection; however, the role of TRIM21 in endothelial dysfunction and lung injury is yet to be defined.

In this issue of the *Journal*, the study by Li and colleagues (pp. 776–785) provides evidence that TRIM21 plays a role in LPS-mediated lung inflammation and endothelial dysfunction (16). They show that overexpression of TRIM21 before LPS challenge effectively reduced LPS-mediated neutrophil influx, cytokine release, and pulmonary edema in mice. Overexpression of TRIM21 in primary human lung microvascular endothelial cells resulted in reduced LPS-induced NF-κB activation, expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), monocyte adhesion, and IL-8 release. Furthermore, in mice challenged with LPS or *Pseudomonas aeruginosa*, and in lung endothelial cells exposed to LPS or TNF-α, there was a significant reduction in TRIM21 expression, which was attributed to increased monoubiquitination and lysosomal degradation in response to an inflammatory stimulus. *In vitro*, overexpression of TRIM21 attenuated LPS-induced increases in endothelial permeability in lung microvascular endothelial cells.

Although this study provides the first evidence in support of a protective role of TRIM21 in ameliorating endothelial barrier dysfunction resulting from endotoxin-induced inflammation, it also has some limitations. The study raises new questions that need to be answered to advance our current understanding of the role of ubiquitin ligase(s) in endothelial barrier function. The clinical relevance of TRIM21 is unclear, as the expression levels of TRIM21 in lung specimens from patients with sepsis or *P. aeruginosa*

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infection have not been investigated and thus cannot be correlated with the data collected from comparable animal model studies. As shown here, LPS-modulated TRIM21 expression was apparent in primary lung endothelial cells, but not in murine lung epithelial (MLE-12) cells. Further characterization of TRIM21 expression and its role in primary alveolar type II cells in LPS-challenged mice would provide insight into the involvement of epithelial ubiquitin-proteasome systems in pulmonary inflammation. The mechanism(s) of TRIM21-dependent regulation of NF-κB activation and expression of ICAM-1 and VCAM-1 in the endothelium by LPS, and how LPS signaling via Toll-like receptor 2 (TLR2) or Toll-like receptor 4 (TLR4) modulates TRIM21 activity, need to be detailed. To date, more than 1,000 E3 ligases have been identified in mammalian cells, and earlier studies from this group provided new insights into the SCF (Skp1-Cullin-F box) component in normal physiology and pathophysiology of lung disorders (17). Unlike TRIM21 overexpression conferring protection in the endothelium, inhibition of the E3 ubiquitin ligase F-box component Fbxo3 using a small-molecule inhibitor effectively ameliorated the severity of viral pneumonia, septic shock, and cytokine-driven inflammation in murine models (18), suggesting a differential role for SCF protein(s) in pulmonary inflammation. Furthermore, different E3 ubiquitin ligases may have similar or opposing effects on regulation of endothelial barrier function, which warrants an in-depth investigation. Thus, pharmacological targeting of specific E3 ubiquitin ligases may be of significant use in combating endothelial dysfunction and lung inflammatory injury as seen in ALI/acute respiratory distress syndrome and its more severe form in sepsis. ■

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Viswanathan Natarajan, Ph.D.
Department of Pharmacology
and
Department of Medicine
University of Illinois
Chicago, Illinois

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