Paradoxical Use of Tumor Necrosis Factor in Treating Pulmonary Edema

In the normal lung, alveolar fluid clearance is tightly regulated, but in disease states, increased alveolar fluid can arise through alterations in Starling forces, including hydrostatic fluid forces (high-pressure edema), increased filtration coefficient, or reduced reflection coefficient, leading to epithelial permeability (low-pressure) edema. Regardless of the cause, alveolar edema elicits mechanical (decreased lung compliance) and physiologic (hypoxemia) perturbations that can be disastrous for a patient, leading to respiratory failure.

Most research in this important area has focused on the etiology and pathophysiology of acute lung injury (ALI)/acute respiratory distress syndrome (ARDS), which occurs when an infectious or traumatic event (pulmonary or extrapulmonary) causes release of inflammatory mediators and neutrophil accumulation that affects the microcirculation and alveolar epithelial layer of the lung, leading to pulmonary edema. ALI may also occur as a result of ischemia-reperfusion injury after lung transplantation and, occasionally, cardiopulmonary bypass.

The cornerstone of therapies addressing low-pressure pulmonary edema focuses on altering hydrostatic forces, using diuretics and positive end-expiratory pressure. In the National Institutes of Health/ARDS Network-funded multicenter Fluids and Catheters Treatment Trial (FACTT), conservative compared with liberal fluid administration in patients with ALI/ARDS with the intent to limit pulmonary edemagenesis increased the number of days free from mechanical ventilation. Survival was similar with both approaches, but survivors managed using the conservative approach were liberated from the mechanical ventilator 3.2 days sooner. Matthay and others reported that in patients with ALI/ARDS, mean alveolar fluid clearance is only 6% per hour compared with 13% per hour in patients with hydrostatic edema (1). A comparison of clinical management strategies showed that reabsorption of pulmonary edema fluid from the alveolar space is necessary for resolution of ALI/ARDS, and that those patients with better maximal fluid clearance have lower mortality and spend less time on mechanical ventilation (2). Simply put, a dry lung is a happy lung.

Absorption of Na^+ ions via apical epithelial sodium channels (ENaC) is essential for water homeostasis in the lung. Transepithelial Na^+ transport initiated at the apical surface by ENaC and accomplished by Na^+/K^+-ATPase drives water absorption from the alveolar surface. Thus, activation of ENaC provides an important therapeutic strategy for restoring lung water homeostasis. Toward this end, it is known that activation of ENaC is regulated by cyclic AMP. Initial findings from the Beta-Agonist Lung Injury Trial (BALTI1) (3) suggested that enhanced intracellular cyclic AMP, stimulated by intravenous salbutamol (albuterol), accelerates the resolution of alveolar edema in adult patients with ALI/ARDS. However, a follow-up trial (BALTI2) (4) was stopped early because of safety concerns, noting increased mortality in the treatment group. In a more recent randomized, placebo-controlled clinical trial on more than 280 patients with ALI/ARDS, aerosolized albuterol was found to be ineffective in improving clinical outcomes (5). In addition, because of its off-target effects, albuterol enhancement of intracellular cyclic AMP may not be the best therapeutic approach to promote ENaC activation in patients with ALI/ARDS.

Might there be another way to increase ENaC activity? With respect to the pathophysiology of ALI/ARDS, we often think of inflammatory cytokines such as tumor necrosis factor α (TNF-α) as central culprits in mediating the negative effects of inflammation, and thus, as part of the pathophysiological sequelae serving to worsen the symptoms of ARDS. Levels of TNF-α are elevated in patients with ARDS, and both activation of TNF-α receptors in the pulmonary microvasculature (endothelial and smooth muscle cells) and alveolar epithelial cells appear to mediate, at least in part, the dysregulation of alveolar fluid clearance and increased microvascular permeability underlying pulmonary edema in ARDS. Previously, the lectin-like domain of TNF-α was shown to activate ENaC in type 2 alveolar epithelial cells (6). Importantly, this effect has been mimicked by a small 17-amino acid circular peptide known as TNF inhibitory peptide (TIP; also named AP301). A recent European study compared placebo treatment with TIP inhalation in patients with ALI/ARDS and found that TIP elicited earlier and more pronounced clearance of pulmonary edema (7, 8). The molecular mechanism underlying TIP-induced ENaC activation remained uncertain until the study by Czikora and colleagues, published in the September 1, 2014, issue of the *Journal* (9).

Their study provides evidence for a novel, nonreceptor-mediated mechanism by which the TIP activates ENaC, and thereby promotes lung alveolar fluid clearance. Specifically, these investigators systematically explored the effects of a synthetic TIP peptide, which mimics the lectin-like (non-TNF-α receptor) domain of TNF-α. In a series of experiments employing a variety of techniques (transgenic triple mTNF knock-in mice expressing mutant TNF-α, molecular biology, biochemistry, and electrophysiology), the investigators provide convincing evidence for a TNF-α (TIP)-mediated mechanism for direct ENaC activation and rectification of both hydrostatic and permeability-based pulmonary edema. They show that TIP directly binds to the intracellular carboxy-terminal of the α subunit of ENaC, thereby increasing the channel open probability and enhancing Na^+ absorption.

They also show that TIP binding maintains expression of the α subunit of ENaC and stabilizes the channel structure through interactions with myristoylated alanine-rich C-kinase substrate and phosphatidylinositol 4,5-bisphosphate that are essential for preserving the open configuration in the presence of pore-forming bacterial toxins.

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Finally, they developed a novel triple mTNF knock-in mouse model, in which "triple" refers to the substitution of nonsense nucleotides encoding for three distinct amino acids that are normally required for the Na^+ uptake stimulatory activity of TNF-α, the so-called functional alveolar liquid clearance-stimulatory domain. The authors show that when these triple mTNF knock-in mice were exposed to pneumococcal cholesterol binding pore-forming toxin, a model of pulmonary edema, there was no change in the quantal generation of TNF-α in the bronchoalveolar lavage fluid, but there was reduced ENaC activity, decreased ENaC-α protein expression, and greater lung edema. This finding suggests a physiological role for the lectin-like domain of native TNF-α in alveolar fluid clearance and the resolution of pulmonary edema. Taken together, these basic science results provide new physiological insight into the potential role of the lectin-like domain of TNF-α and support the novel therapeutic use of TIP aerosols in patients with ALI/ARDS and ischemia reperfusion lung injury. ■

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Searching for Distinct Mechanisms in Eosinophilic and Noneosinophilic Airway Inflammation

Chronic rhinosinusitis (CRS) is an inflammatory disease of the upper respiratory tract affecting up to 30 million Americans annually. It is associated with a significant impairment in quality of life and places a large financial burden on the healthcare system, with more than $6 billion spent annually on management (1–4). CRSwNP, a subset of CRS, is characterized by the presence of nasal polyps and chronic inflammation of the sinonasal mucosa. In European and American patients, CRSwNP is characterized by type 2 inflammation and eosinophilia. However, there is accumulating evidence, especially in China, that almost half of patients with CRSwNP in Asian countries have a noneosinophilic pattern of inflammation in their polyp tissue that is characterized by a mixed type 1 and/or type 3 response (5, 6). Although the mechanisms that drive these phenotypes are unclear, it has been suggested that differences in Th cell subsets found in polyps from eosinophilic and noneosinophilic patients may play an important role (7). Dendritic cells (DCs) are known to be important in skewing Th responses in the mucosa (8), and thus may be important for skewing Th cells in polyps. However, there has been a lack of in-depth analyses of Th cell subsets found in polyps from different CRSwNP groups, and few studies have investigated the importance of DCs in CRSwNP pathogenesis (9, 10).

In this issue of the *Journal* (pp. 628–638), Shi and colleagues evaluated the function and phenotype of Th and DC subsets from polyps of eosinophilic and noneosinophilic patients with CRSwNP in China to assess any differences (11). Interestingly, many of the features examined in the Th and DC subsets isolated from polyps did not differ between the two groups of patients with CRSwNP. The researchers found similar elevations of $IL\text{-}17A^+$ and $IFN\text{-}\gamma^+$ $CD4^+$ cells in polyps from both groups compared with controls, confirming a recent study from Europe (12). Likewise, they found similar elevations of activated DC subsets (both myeloid DC [mDC] and plasmacytoid DC [pDC]), and these DCs produced equivalent elevated levels of IL-6 and