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TGF- β and lung fluid balance in ARDS

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Acute respiratory distress syndrome (ARDS) is characterized by hypoxemia from flooding of the distal airspaces of the lung with protein-rich edema fluid. Pulmonary edema develops because of an increase in lung vascular permeability and from injury to the alveolar epithelium that diminishes the normal capacity of the alveolar epithelium to remove edema fluid from the airspaces (alveolar fluid clearance, AFC) (1, 2). The severity of alveolar edema depends on the competing effects of increased permeability and the active clearance of edema fluid from the airspaces in regions where the epithelium is intact. The vectorial transport of sodium into the interstitial space provides an osmotic gradient for absorption of water. The sodium concentration gradient is established by Na⁺/K⁺-ATPase located in the basolateral membrane of polarized alveolar epithelial type 1 and type 2 cells (Fig. 1). Trans-epithelial sodium transport also requires an adequate paracellular barrier, apically polarized sodium channels such as ENaC (the epithelial sodium channel), and chloride transport (3, 4).

In PNAS, Peters et al. (5) report a unique signaling pathway by which TGF- β acutely reduces transepithelial sodium transport by inducing endocytosis of ENaC. Targeted effects on the ENaC-dependent sodium current are important to AFC because conductance through this channel accounts for a large fraction of the total transepithelial sodium transport. The multifunctional TGF- β cytokine family has detrimental effects on both lung permeability and AFC; however, the mechanisms for TGF- β -mediated epithelial barrier dysfunction are not completely understood. Using studies in rabbits, ex vivo rabbit lungs, mice, clinical fluid and tissue samples, and cell-based studies, these investigators provide evidence for the adverse effect of TGF- β on lung fluid balance and AFC. Based on data from inhibitor-based and genetic approaches, a TGF- β -dependent signaling pathway is identified. The data are consistent with a pathway that places TGF- β -dependent SMAD2/3 phosphorylation proximal to phospholipase 1 activation and NAPDH oxidase 4 (NOX4)-dependent reactive oxygen species generation.

Data from amino acid substitution experiments support the hypothesis that oxidation of the conserved cysteine at position 43 of the β -subunit of ENaC acts as a signal for internalization. This oxidation event appears to require NOX4. Consistent with this hypothesis, NOX4-null mice demonstrated preserved lung fluid balance in the TGF- β -dependent bleomycin lung injury model; however, differences in lung injury severity rather than direct effects on ENaC transport are also possible to explain the results, and lung fluid transport rates were not measured in these mouse studies. Finally, oxidation-dependent endocytosis of ENaC mediated by TGF- β does not appear to involve the previously described regulator of ENaC ubiquitination neural precursor cell expressed, developmentally down-regulated (Nedd)4-2 (6).

Over the past two decades, details of the mechanisms for sodium and fluid transport in the alveolar epithelium have emerged (3, 4, 7). Na⁺/K⁺-ATPase is the driver of AFC and a variety of regulators of pump function have been described (7). Among these regulators are the β 2-adrenergic and dopamine receptor pathways, which act through the second messenger cAMP and other mechanisms to increase pump activity and protein localization to the basolateral membrane. In addition, growth factors and hormones enhance transepithelial sodium conductance through effects on Na⁺/K⁺-ATPase. FX/YD family proteins, such as the γ -subunit of Na⁺/K⁺-ATPase, also promote Na⁺/K⁺-ATPase stability in the membrane and bidirectionally regulate pump activity (8, 9).

Apical membrane sodium conductance occurs through several sodium channels, but the major contributor is ENaC. Although the proportion of ENaC (amiloride sensitive) sodium conductance varies with species, ENaC is a major contributor in human type 2 cells. Many of the factors that increase Na⁺/K⁺-ATPase activity also increase ENaC activity. Negative regulators of ENaC include purinergic P2Y receptor activation (10), nitric oxide (11, 12), innate immune mediators (13), hypoxia (14), and TGF- β (15). Chloride transport

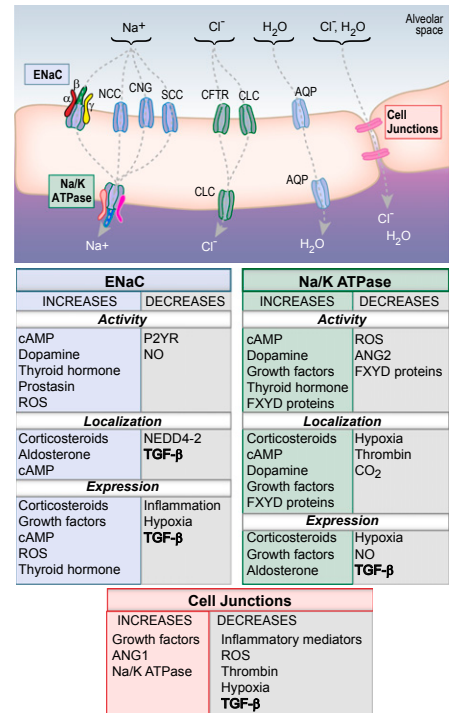


Fig. 1. Lung fluid balance in ARDS. Removal of edema fluid from the alveolus requires a sodium concentration gradient established by Na⁺/K⁺-ATPase. ENaC is a key pathway for sodium conductance through the apical membrane of polarized alveolar epithelial cells, but other sodium channels, including nonselective cation channels (NCC), cyclic nucleotide gated channels (CNG), and highly selective cation channels (SCC) make contributions. Cyclic-AMP-dependent and -independent pathways increase sodium transport. A primary c-AMP-dependent pathway is β 2 adrenergic receptor activation. Hormones and growth factors also increase sodium transport. Reactive oxygen species (ROS), nitric oxide (NO), and purinergic receptors (P2YR) negatively regulate sodium transport. Chloride transport occurs through CFTR and other chloride channels (CLC). Water traverses the epithelium through the paracellular pathway and through aquaporin channels (AQP), although genetic knockouts of AQP does not slow fluid clearance. Factors that affect cell junctions and paracellular permeability play an important role in lung fluid balance. (Figure design by Diana Lim.)

also influences AFC, particularly at high rates of sodium transport (16, 17).

The pathway reported by Peters et al. (5) is rapidly activated and appears independent of transcriptional regulation. Prior studies support

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more canonical TGF- β -mediated changes in mRNA expression occurring over many hours or days. For example, prolonged exposure to TGF- β induces sustained decreases in the expression of the α -subunit of ENaC (15). Based on data from clinical samples in this study, a sustained increase in TGF- β activity may occur in the airspaces of patients with ARDS. Evaluation of clinical samples in the present study showed that ENaC mRNA levels were similar in lung samples from patients with or without ARDS, but because the sample size is limited (nine samples), it remains possible that ENaC expression is different in ARDS patients. Thus, the impact of the TGF- β -dependent effects on ENaC trafficking on lung fluid balance in ARDS patients is unclear. Data from the present study support the possibility that inhibition of TGF- β could be a potential therapeutic target, but transcriptionally mediated effects of TGF- β receptor activation may not be acutely reversible by subsequent TGF- β inhibition. Nevertheless, the regulation of ENaC trafficking via oxidation of cysteine 43 may represent a promising target for further study.

Alveolar barrier dysfunction in ARDS leads to more pulmonary edema and to the systemic release of biological mediators from the lung, contributing to the failure of other organs (18). Therefore, the value of therapies targeted primarily at augmenting epithelial sodium and fluid transport across the alveolar epithelium may be limited, particularly if the alveolar epithelial barrier has

been denuded from apoptosis and necrosis of alveolar epithelial cells (1, 3, 19). Based on preclinical studies, β -adrenergic agonists accelerate AFC in the lung (3), but recent clinical ARDS trials found no benefit on patient outcomes (1). Restoration of an intact alveolar epithelium may be required to improve both edema fluid clearance and clinical outcomes in ARDS patients.

Targeting the TGF- β pathway as an ARDS therapy has rationale because TGF- β appears

to mediate both increased permeability and decreased AFC. However, TGF- β plays an important role in regulating inflammation, immunity, and tissue repair in several organs, thus systemic inhibition of TGF- β may have adverse effects. Nevertheless, as Peters et al. (5) propose, insights into the molecular mechanisms responsible for the detrimental effects of TGF- β on alveolar barrier function could lead to a new ARDS therapeutic that would not require global inhibition of TGF- β .

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