Lung Biology Research & Trainee Day
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Category: Predoc
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Title: Gene Transfer of MRCKα Rescues Lipopolysaccharide-Induced Acute Lung Injury
Abstract: Gene Transfer of MRCKα Rescues Lipopolysaccharide-Induced Acute Lung Injury Jing Liu, Michael Barravecchia, and David A. Dean Rationale: Acute Respiratory Distress Syndrome (ARDS) is a severe condition which is characterized by significant alveolar fluid accumulation and insufficient gas exchange, which ultimately leads to acute respiratory failure. Underlying ARDS, there is impaired alveolar fluid clearance (AFC) and disrupted alveolar-capillary barrier. Therefore, enhancing AFC and repairing the alveolar-capillary barrier are considered therapeutic goals for gene delivery for ARDS. We previously have shown that overexpression of the β1 subunit of the Na+, K+-ATPase (β1- Na+, K+-ATPase) increases AFC in healthy and injured mouse and rat lungs. We have also found that electroporation-mediated gene delivery of the β1- Na+, K+-ATPase rescues lipopolysaccharide (LPS) induced acute lung injury (ALI) by upregulating tight junction (TJ) proteins and pulmonary barrier function, demonstrated by decreased lung permeability, total protein and cellularity in bronchoalveolar lavage (BAL) fluid, and improved overall outcome of lung injury. While studying how the β1-Na+, K+-ATPase signals to and increases lung barrier function, we identified MRCKα (CDC42 binding protein kinase alpha) as an interacting partner of the β1 subunit by mass spectrometry. MRCKα is a downstream effector of CDC42, a small GTPase, and is involved in multiple cell behaviors, including junction formation. Previous data indicate that MRCKα mediated β1's upregulation of TJ proteins and epithelial barrier integrity in cultured cells. However, it is unknown whether MRCKα can upregulate pulmonary barrier function and treat LPS induced ALI in vivo. Understanding this question would help to determine the therapeutic potential of MRCKα for ARDS. This study tested whether electroporation-mediated gene transfer of MRCKα could treat LPS induced ALI in vivo. Methods: Plasmids expressing MRCKα or β1-Na+, K+-ATPase were delivered individually or in combination by aspiration and transthoracic electroporation to mice which had been pre-injured by LPS intratracheal administration 24 hours earlier. Two days after gene delivery, various endpoint assays were performed to evaluate lung edema, permeability, inflammation and histological injury. Results and conclusions: Overexpression of MRCKα alone or in combination with β1- Na+, K+-ATPase attenuated LPS-increased Wet/Dry ratio, lung leakage, and BAL cellularity and protein concentration, restored TJ protein expression, and improved overall outcome of lung injury. In addition, we found that gene transfer of MRCKα alone did not enhance AFC, indicating its role in regulating alveolar capillary barrier integrity without promoting ion transport. These results indicate that MRCKα could benefit the pre-injured lungs by reducing pulmonary edema, restoring lung barrier function and reducing inflammation. Moreover, they also suggest that improving barrier function alone may be of equal or even more benefit than improving AFC in order to treat ALI/ARDS.
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