Amplification Loop Between Mitochondria and Complement in ARDS

Hrishikesh S. Kulkarni, M.D. (Year 2)

Abstract

The overarching goal of this proposal is to enhance both survival and quality of life after acute respiratory distress syndrome (ARDS), a major cause of mortality. Infectious causes of ARDS include pneumonia, and sterile causes include aspiration and ischemia-reperfusion injury after lung transplantation. However, the immune-mediated changes that incite and propagate acute lung injury (the pathological correlate of ARDS) are largely unknown. We and others have reported that circulating levels of complement proteins – a component of our innate immune response – are associated with improved survival during critical illness due to ARDS.

In the first year of this grant, we showed that C3 is critical for protection against stress-induced epithelial cell death using both *in vitro* and *in vivo* approaches. These observations hold true in both infectious as well as sterile injury, suggesting that the cytoprotective effects of C3 are not entirely dependent on pathogen clearance. Additionally, we demonstrated that an intracellular protein – complement Factor B – is necessary for the cytoprotective effects of C3. Data generated from this grant's first year show that the absence of C3 in airway epithelial cells results in decreased mitochondrial function. What remains unclear is if the cytoprotective effects of C3 are due to the impaired clearance of mitochondria in the absence of C3 (i.e., impaired mitophagy).

To address this question, we need to first answer if damaged mitochondria promote complement activation, and subsequently evaluate whether complement facilitates mitophagy in airway epithelial cells. Thus, our specific aims for the second year of funding remain the same:

- Aim 1: dissect pathways by which mitochondria released from the lung during injury activate complement, and
- Aim 2: assess how complement modulates mitochondrial respiration and homeostasis during lung epithelial injury.

As part of Aim 1, we will address if mitochondria derived from human lung epithelial cells activate complement, and will identify the predominant pathways by which they activate the complement cascade (i.e., classical, lectin, or alternative). As part of Aim 2, we will assess how complement modulates cellular mitochondrial activity during lung epithelial injury. Utilizing live-cell imaging and flow cytometry of human lung epithelial cells, we will determine how the absence of intracellular complement proteins affects mitochondrial function and mitophagy in the setting of sterile (i.e., oxidative stress, acid) and infectious (i.e., bacteria-induced) injury. This approach will then be extrapolated to *ex vivo* models of lung injury using human lung tissue. Hence, through this proposal, we will decipher the mechanisms by which prognosticators of ARDS disease progression—identified by us and others—promote lung epithelial function, with the ultimate goal of mitigating severe lung disease.