Dr. Jahar Bhattacharya

Significance. Acute Lung Injury (ALI) is a major cause of mortality and morbidity. Pharmacospecific therapies are not available. Since ALI develops rapidly, fast-acting pharmacospecific therapy is required. We propose that loading lung endothelium with a purified, phosphorylated form of focal adhesion kinase (FAKp), given in non-covalent conjugation with the cell permeable peptide, TAT, will protect against ALI by inducing barrier enhancing protein-protein interactions. Since reported strategies for inhibiting FAK are based on non-specific small molecules that induce non-specific effects, our TAT-FAKp strategy will provide specific understanding of FAK's barrier-strengthening effect.

Proposal. To test the hypothesis that activated FAK strengthens the endothelial barrier, a purified, activated form of FAK conjugated with the cell-permeable peptide, TAT, will be loaded in lung endothelia. It is expected that this conjugated constructed, called here TAT-FAKp will induce protein-protein interactions between FAK, paxillin, vinculin and α-actinin-1 to form a protein complex, called here the FPVA complex, that enhances Factin at adherens junctions, leading to cadherin clustering and barrier enhancement. This hypothesis will be tested in the context of barrier quantifications in cultured monolayers and animal models of ALI, using strategies for by interfering with FPVA forming protein-protein interactions. These strategies will be (1) inducing point mutations on FAK tyrosine residues, and (2) expressing mutations on the binding domains of the partner proteins of the FPVA complex.

Approach. Specific Aims 1 and 2 will evaluate the barrier protective protein-protein interactions induced by TAT-FAKp in endothelial monolayers and mouse lungs. These aims will be achieved through expression of mutants in cultured human lung microvascular, pulmonary artery endothelial cells, and in mouse lungs. In the presence of these mutations, protein–protein interactions and cadherin stability will be determined as reflected by studies of fluorescence recovery after photo bleaching and by assays of endothelial permeability as reflected by the filtration coefficient (*Kf),*lung water in mice, point permeability assays and the transendothelial resistance (TER), transmigration of leukocytes, in cultured ECs. We will determine protective effects through blinded mouse survival studies.

Expected Outcome. Based on supportive preliminary data, we expect to show that loading endothelia with TAT-FAKp will abrogate thrombin or oxidant-induced hyperpermeability in lung endothelial monolayers, and ALI-induced lung hyperpermeability in mice. We expect that mouse survival following ALI will be considerably more extended in TAT-FAKp-treated than in untreated mice. For the first time, the positive therapeutic effects TAT-FAKp will be understood in terms of critical protein-protein interactions induced by activated FAK.