Staphylococcal Activation Of EGFR Facilitates Invasion Across The Pulmonary Mucosal Barrier

G. Soong, BS[1], J. Chun, PhD[2], F. Martin, PhD[2], T.S. Cohen, MS[3], A.S. Prince, MD[1]
[1]Columbia University - New York, NY/US, [2]Columbia University - New York/US, [3]University of Pennsylvania - Philadelphia, PA/US

RATIONALE: *Staphylococcus aureus* are an important cause of severe pneumonia especially in hospital settings and as a potentially fatal complication of influenza. Nasal colonization with *S. aureus* increases the likelihood of infection, yet is unclear how mucosal organisms once aspirated, cross an intact epithelial barrier. The staphylococcal surface protein, protein A (SpA), binds EGFR and activates several signaling cascades. EGFR has a central role in cell development and motility and is linked to both actin and E-cadherin. EGFR initiated MAPK activity is linked to activation of Rho/ROCK/ MLC induced actinomyosin contraction and to epithelial proteases.

METHODS: We postulated that staphylococci could stimulate contraction of the epithelial cytoskeleton as well as cleavage of junctional proteins, such as E-cadherin and occludin, thus creating a gap in the paracellular junctions sufficient to enable staphylococcal translocation.

RESULTS: Using strain Newman and the epidemic USA300 MRSA strain of *S. aureus* and isogenic *spa* null mutants, we demonstrate disruption of the actin cytoskeleton in polarized human airway monolayers in response to *S. aureus* strains expressing *spa*, but not the mutants unless complemented with the SpA peptide. Bacterial translocation and permeability to 10k MW fluorescent dextran similarly was limited to SpA+ strains and correlated with SpA+ induced calpain activity. SpA- exposed monolayers had activated RhoA and phosphorylated MLC. The SpA+ strains also induced cleavage of the extracellular portions of E-cadherin and occludin that span the paracellular junctions. Monolayers treated with EGFR inhibitor BPDQ, the ERK inhibitor U0126, the ROCK inhibitor Y27632 or the calpain inhibitor calpeptin had significantly ($p<0.05$) decreased staphylococcal transmigration; by confocal imaging these monolayers were found to be protected from staphylococcal induced disruption of the cytoskeleton. In a mouse model of staphylococcal pneumonia, mice pretreated with the EGFR inhibitor AG14578 had significantly less bacteremia than did the untreated controls ($p=0.017$).

CONCLUSION: These studies suggest that activation of the EGFR- ERK/RhoA/MLC and EGFR-ERK calpain pathways by *S. aureus* protein A enable these non-motile organisms to pass through the paracellular junctions and gain access to the subepithelia space and disseminate into the bloodstream. Thus in addition to the other potent virulence factors of *S. aureus*, the protein A- EGFR interaction facilitates staphylococcal invasion through epithelial and likely through endothelial barriers as well.