

University of North Dakota**Lung Epithelial Cells in Pseudomonas Infection****ABSTRACT I**

We would like to request some human tissues for our study. The purpose of this study is to determine whether *Pseudomonas aeruginosa* (PA) infects and enters to lung epithelial cells. It is unclear whether PA can infect and get to inside of lung epithelial cells in patients with Cystic Fibrosis (CF) as PA mainly invades airway space. Particularly, no previous study has been done in assessing the lung infection with PA in human tissues. Study Procedures: We plan to make cryostat sections (frozen tissue sections) with the tissues that will be obtained from the National LTRC (NIH consortium). The tissues include up to 10 lung tissues from CF patients, 10 lung tissues from normal donors. We will detect whether PA can internalize into the lung epithelial cells using histochemistry method that will stain the tissue using antibody against PA and visualized with optical microscopy. Statistical analysis of the data will demonstrate that the chance (likelihood) of PA enter to the lung epithelial cells. Standard markers for lung type II cells (surfactant protein A) will be stained to look for colocalization (overlapping positioning) of PA with the cells. The second experiment will determine the internalization of PA by immunofluorescence with the same antibody against PA, and results will be viewed using fluorescence microscopy. The third experiment will use transmission electron microscopy to test whether PA can enter to the lung epithelial cells. The final method is the most convincing and direct measurement of the PA invasion by an electron microscopy with specific antibody against PA. We will finally check the cytokine secretion manners in these samples. If no CF patients available we could use other COPD sample to look at the cytokine production manner with AECII cells. Our lab has been approved for infectious study with PA in vitro and in vivo. The experimental procedures must be done following the guidelines of NIH for proper handling of human tissues to avoid contamination and other risks. Personnel including the PI and graduate student (Huang Huang) have successfully completed the training of human subject research.