

Biomedical Research Funded in 2006

Elizabeth Nash Memorial Fellowship Program

- 1) George Caughey, MD, Principal Investigator - University of California, San Francisco
Xiang Xu, MD, PhD – Post Doctoral Fellow (Second year funding)

“Probing the role of Hepatocyte Growth Factor (HGF) regulation by neutrophil secretions”

The study on the regulation of CF epithelial function by neutrophil peptidase-generated antagonists of HGF: Hepatocyte growth factor (HGF) is essential for epithelial development and repair. HGF can be induced in damaged tissue including the lung, and has been shown in an animal model to decrease airway inflammation and remodeling, e.g. fibrosis. Preliminary studies for this project have found that enzymes released during inflammation in the CF lung inactivated HGF by cleaving the protein. Furthermore, this cleavage of HGF produces a fragment that functions to oppose epithelial repair. The goal of this project is to further understand the function of these HGF fragments generated by cleavage and their effect on CF epithelial cell function. This study will provide insight into the chronic epithelial wounding seen in CF and may identify strategies to limit this airway damage.

Amount Funded: \$40,000.00

- 2) Ron Kopito, PhD, Principal Investigator – Stanford University, and John Christianson, PhD, Post Doctoral Fellow (Second year funding)

“Elucidation of the Ubiquitin Pathway involved in degradation of delta F508 CFTR”

This project focuses on the identification of genes involved in degradation of CFTR Δ F508: All cells have a quality control check point for the synthesis of proteins. Proteins that do not fold properly are recognized by the cell as misfolded and subsequently these proteins are degraded by the cell. How the cell carries out this quality control check and the proteins involved in this process is not clearly understood. The goal of this project is to use a fluorescent-based screening assay to identify specific proteins involved in the recognition of the misfolded CFTR Δ F508 protein. The identification of these specific proteins for mutant CFTR will become the target of a rationale therapeutic design to enhance CFTR maturation.

Amount Funded: \$40,000

- 3) Jeffrey Wine, PhD, Principal Investigator - Stanford University, and Jae Young Choi, MD, PhD Post Doctoral Fellow (Second year funding)

“Development of methods to test longevity of mucus plugs in airway glands in CF and imaging of glandular secretion and single-cell secretion”

This project studies the mucus structure in normal, CF and pharmacologically treated airways: The goal of this project is to investigate if airway gland dysfunction plays a role in CF lung disease. The role of the airway gland in CF has become controversial. The findings published by Boucher and colleagues from the University of North Carolina, Chapel Hill show that CFTR protein expression in the airway gland is significantly lower than previously reported. Mucous secretion is important for normal lung function, and is altered in CF lungs. Thus the question of the role of airway glands in CF lungs needs to be understood in order to identify important treatments to combat CF lung disease.

Amount Funded: \$40,000

- 4) Beate Illek, PhD, Principal Investigator - Children's Hospital Oakland Research Institute, Eun Jin Kim, PhD, Post Doctoral Fellow (Second year funding)

"Vitamin C Uptake into Cystic Fibrosis Airways"

This study is on role of vitamin C on CFTR transport and *Pseudomonas aeruginosa*-induced oxidative stress in cystic fibrosis airways: Vitamin C is a normal component of the airway, and concentrations of this natural antioxidant are decreased during oxidative stress - a condition that occurs in the CF lung. Interestingly, Dr. Illek and colleagues have demonstrated that vitamin C activates both the wild type and the corrected mutant CFTR protein. Thus, the goal of this project is to determine the beneficial effects of vitamin C in protecting the airways from pyocyanin-induced inhibition of CFTR channel activity. In other words, in the presence of a *Pseudomonas* infection, will vitamin C and/or other antioxidants protect the airways and preserve CFTR channel activity? This study will have a direct application to the design of future preclinical studies.

Amount Funded: \$40,000.00

New Horizons Research

- 5) Jonathan Widdicombe, PhD, Principal Investigator - University of California, Davis

"CFTR vs Ca-activated chloride channels in airway glands"

The relative importance of CFTR vs. Ca-activated chloride channels in the production of airway gland liquid secretions by serous cells is becoming increasingly controversial. This is largely because CFTR is a very scarce protein, and attempts to localize it using biochemistry have been difficult and produced conflicting results. Measurements of function may therefore prove more informative. Cells in glands are either serous (water-secreting) or mucous-secreting, with the water secreted by the serous cells flushing mucus from the glands. We have previously shown that gland cell cultures of mixed seromucous phenotype have much higher levels of both CFTR and Ca-activated chloride channels in their apical membranes than do mucous cultures. This was to be expected because secretion of water by epithelia in general is down osmotic gradients generated by active secretion of chloride. Recently we have developed cultures of markedly serous phenotype as revealed by specific cell markers and by their ultrastructure. We expect both CFTR and Ca-activated chloride channels to be further increased in the serous cultures as compared to the seromucous cells. To test this hypothesis, we will use various physiological and pharmacological techniques. We will then identify the specific Ca-dependent chloride channels present by a variety of techniques including physiological, pharmacological and molecular biology.

Since CFTR is found in both the surface epithelium and glands, knowledge of the relative contributions of Ca-dependent chloride channels vs. CFTR in the production of gland liquid secretions will shed light on the relative importance of airway surface and gland epithelia in the pathogenesis of cystic fibrosis.

Amount Funded: \$59,552.00

6) Charles Falany, PhD, Principal Investigator - University of Alabama at Birmingham

“Estrogen Sulfation in the Dysregulation of Hepatocyte Growth Hormone Signaling in Cystic Fibrosis”

Cystic fibrosis (CF) is primarily associated with breathing problems and lung congestion; however, it also affects other organs including the liver. Many CF patients have growth deficiencies and require treatments to increase growth and weight gain. Studies indicate that growth problems are related to livers of CF patients not responding properly to a hormone called growth hormone (GH).

Growth hormone stimulates the liver to increase the synthesis of another hormone called IGF-1. IGF-1 circulates in the blood and is important in stimulating proper bone and tissue growth. Many CF patients with growth problems have decreased IGF-1 levels in their blood.

Studies in CF mice and human liver cells suggest that active estrogens such as β -estradiol (E2) must be present in the liver for GH to work properly. These studies suggest that an enzyme called sulfotransferase (SULT) 1E1 is increased in the livers of CF patients. SULT1E1 is important in metabolizing and inactivating the estrogen E2. High SULT1E1 activity prevents GH from working properly.

Liver SULT1E1 activity is high in the first part of fetal development and decreases to low adult levels at birth. In many CF patients, SULT 1E1 levels may not be decreasing properly so that more E2 is inactivated in the liver than is optimal and thus GH cannot stimulate the correct amount of IGF-1 production. This would result in growth deficiencies in CF kids. We propose to investigate whether this increase in SULT1E1 is involved with inhibiting IGF-1 synthesis in human liver cells and CF patients.

Amount Funded: \$60,000.00

7) Isabel Virella-Lowell, MD, - Medical University of South Carolina

“Loss of Acid Sphingomyelinase Induction by Pseudomonas Aeruginosa in Cystic Fibrosis”

The outer covering of the cell and the small platforms that protrude from it are where early interactions between cells and invading bacteria occur. Recently, these platforms have been found to have an important role in the body's defense against *Pseudomonas*, the bacteria most commonly found in the airways of CF patients. These platforms are thought to be involved in bringing the bacteria into the cells and clearing it from the airway. The interactions between the platforms and bacteria are mediated by chemicals, called enzymes.

This project focuses on the role of a specific enzyme, acidic sphingomyelinase (ASMase). Our data shows that in non-CF cells, ASMase gene levels were elevated when they were infected with *Pseudomonas*. The gene levels for ASMase did not rise with infection in CF cells. Based on this finding, the proposed study looks at whether a defective ASMase response in CF leads to ineffective clearance of *Pseudomonas*.

Initial experiments will be conducted in CF and non-CF airway cells grown in the laboratory to determine the effects of *Pseudomonas* in ASMase production and cell death. We will then investigate whether giving ASMase changes the response of CF mice to *Pseudomonas* infection. In particular, we will look at swelling and inflammation in the airway, cell death, bacterial clearance and survival of the CF mice. Overall, this project looks to define the role of ASMase in bacterial infections in CF and to determine whether supplementing ASMase is a good potential therapeutic strategy for CF.

Amount Funded: \$30,448.00