

Biomedical Research Funded in 2007

New Horizons Research

- 1) Jonathan Widdicombe, PhD, Principal Investigator - University of California, Davis (Second year funding)

“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

- 2) Charles Falany, PhD, Principal Investigator - University of Alabama at Birmingham (Second year funding)

“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

- 3) Isabel Virella-Lowell, MD, - Medical University of South Carolina (Second year funding)

“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

Elizabeth Nash Memorial Fellowship Program

- 4) Jeffrey Wine, PhD, Principal Investigator - Stanford University, and Monel Sonecha, MD, University of California San Francisco, Post Doctoral Fellow

“Clinical and Basic Research Studies of CF Airway Gland Secretions.”

CF involves a defect in a protein (CFTR) present in the airway glands. Airway glands secrete mucus onto the airway surface. Gland mucus is an important component of the defense mechanism against environmental agents (e.g. bacteria) that we inhale. Gland mucus secretion is controlled by local nerve cells in the airways; the local nerve cells are controlled by local stimuli and by central nerves arising from the brain.

The central nerves are stimulated in response to strong irritation, such as inhalation of fluid or food in the airway. They then stimulate glands to secrete through a non-cystic fibrosis protein. This causes the glands to secrete large amount of fluids and also provokes coughing as a defense mechanism to clear the airways. This kind of gland secretion is still strong in cystic fibrosis patients.

In contrast, the local nerves are also weakly stimulated by mild irritating agents, such as inhalation of bacteria or viruses during normal breathing. We hypothesize that the response to mild local stimulation is mediated mainly or entirely by gland secretion that requires CFTR. This 24x7 gland secretion through CFTR allows normal airways to remove inhaled bacteria; thus airways are usually sterile.

In the absence of functioning CFTR, the glands no longer respond to these local stimuli, so bacteria are able to reside in the mucus for much longer times unless they are killed and cleared with inhaled antibiotics and mechanisms to mobilize the intact gland secretion system. Our research seeks to gain control of CF glands so that their residual function can be used to help fight against airways infections.

Amount Funded: \$40,000.00

- 5) Jason Eiserich, PhD, Principal Investigator – University of California Davis, and Vihás Visu, PhD, Post Doctoral Fellow

“Oxidative Stress in Cystic Fibrosis Respiratory Tract Secretions: Therapeutic Strategies”

The purpose of this study is to quantify oxidative processes at respiratory tract surfaces by studies of freshly obtained sputum from adult CF patients, and to assess the capacity of potentially therapeutic anti-oxidants (N-acetyl-L-cysteine [(NAC)], glutathione [(GSH)], ascorbic acid) to modulate these processes. The results will help characterize potential benefits that might be expected to occur subsequent to therapeutic aerosol antioxidant treatments in this disorder known to be associated with oxidative stress.

Amount Funded: \$40,000.00

- 6) Paul Quinton, PhD, Principal Investigator – University of California San Diego, and Ruth Muchekehr, PhD, Post Doctoral Fellow

“Does Poor Bicarbonate Secretion Impair Cervical Mucus Release in CF?”

What causes the thick aggregated mucus found in mucus-producing organs affected by CF? As well as a loss in Cl⁻ conductance, bicarbonate conductance is also lost in CF. Mucins, the main component of mucus, are large negatively charged molecules that are packaged in small vesicles within cells. In order to package these large molecules in small vesicles, positively charged Ca²⁺ and H⁺ ions are required to shield the negatively charged side chains of mucins. Once released from the cell, Ca²⁺ and H⁺ need to be rapidly removed in order for mucins to expand properly and form a mucus gel layer on the cell surface.

We propose that bicarbonate (HCO₃⁻), which readily binds to Ca²⁺ and H⁺, plays a critical role in removing this ‘shielding’ of mucins. Our hypothesis is that in CF, the failure to secrete HCO₃⁻ – along with mucins, reduces the rate and degree of mucin expansion, leading to a final, thick sticky mucus associated with CF.

Amount Funded: \$40,000.00

- 7) Joanne Engel, MD, PhD, Principal Investigator – University of California San Francisco and Iwona Bucior, PhD, Post Doctoral Fellow

“Pseudomonas Binding and Abnormalities in Glycosylation of CF Lung Epithelial Cells.”

Glycosylation, which is the process or result of adding sugars to proteins. Working with Drs. Joanne Engel and Keith Mostor at UCSF, experts in epithelial trafficking, she is investigating the belief that *Pseudomonas* binds to sugars on the proteins at the epithelial cell surface. The purpose of her study is to determine how altered protein glycosylation affects *Pseudomonas* binding.

Amount Funded: \$40,000.00