OCHSNER-UNO COLLABORATIVE UNDERGRADUATE RESEARCH EXPERIENCE (CURE) – 2022 PROJECTS

1) ITR Nephrology Lab - 1 UNO student

Juan C. Velez, MD and Doan Nguyen, PhD

Project title: Identification and validation of novel protein biomarkers in urinary casts to assess kidney function and disease

Description:

Diagnosis of the cause of acute kidney injury has significant clinical implications. As kidney tubules are vulnerable to ischemic and toxic injury, we hypothesize that urinary sediment from patients with acute tubular injury (ATI) contain cast proteins that can be used to establish the diagnosis. Using proteomic approaches, we have analyzed the protein composition of "muddy brown" granular casts and identified surrogate biomarker of ATI.

The student will assist with granular cast purification and microscopy imaging and documentation of patients with ATI. Western blot and immunofluorescent analysis will be carried out to validate the identified protein biomarkers.

2) ITR Neuroscience Lab – 1 UNO student

Ifeanyi Iwuchukwu, MD and Doan Nguyen, PhD

Project title: MicroRNA Regulation of Inflammation in Intracerebral Hemorrhage and Seizure

Description:

Intracerebral hemorrhage (ICH) accounts for 15-20% of all patients with 'acute stroke' and carried a 60%morbidity and mortality. Approximately 15% of patients experience seizure post-ICH. The pathophysiology of post-ICH seizure is not well known, but inflammation-related pathophysiological processes may be involved with onset of seizure. microRNA are recognized as potential biomarkers in neurological diseases and the expression pattern may reflect distinct biological processes and clinical outcome. Our laboratory has identified several miRNA biomarkers that predict seizures after ICH. MiR-4317 was identified as a potential biomarker of post-ICH late seizure and we are investigating the expression levels in whole blood and human

hippocampal tissues. In addition, we will identify their gene targets and the regulatory gene networks associated with inflammation.

Project 1. miR-4317 regulation of SLC38A1, a glutamine-glutamine transporter, in post-ICH late seizure.Expression of miR-4317 is negatively correlated to SLC38A1, a gene target of miR-4317 involved in glutamine transport. The student will assist with tissue sectioning, staining, in situ hybridization and quantification of miR-4317 and SLC38A1 in human hippocampal biopsies.

3) ITR Infectious Disease Lab – 1 UNO student

George Pankey, MD, Director and Debbie Ashcraft, MT(ASCP)SM, Supervisor

Project title: Evaluation of Colistin Broth Disk Elution and ETEST Compared to Broth Microdilution

Description:

The polymyxins (colistin and polymyxin B) are antimicrobial agents of last resort for treating multidrug-resistant (MDR) Gram-negative infections that have emerged over the past decade. There is significant clinical and pharmacokinetic/pharmacodynamics (PK/PD) data available that suggest these agents may have limited efficacy, but their use continues because of a lack of active alternative antimicrobials or a lack of availability and high cost of new antimicrobials with targeted activity against MDR infections. There is a high rate of renal and central nervous system toxicity associated with the use of polymyxins. Therefore, determining the organism's minimum inhibitory concentration (MIC) is extremely valuable when treating with polymyxins.

Unfortunately, many clinical laboratories are unable to perform susceptibility testing for the polymyxins—due to a lack of accurate and reliable methods. The performance of Etest Research Use Only gradient strips has been poor, and the Clinical and Laboratory Standards Institute (CLSI) states that broth microdilution is the only validated method for the polymyxins (which is rarely performed in clinical laboratories). Our Ochsner diagnostic laboratory currently performs colistin susceptibility testing using Etest strips. In 2020, the CLSI (2020) approved a colistin broth elution method which is acceptable compared to the standard broth microdilution method and can be utilized in clinical diagnostic laboratories.

This study will include patients' bacterial isolates (Enterobacterales, Pseudomonas aeruginosa, and Acinetobacter baumannii) collected from Ochsner Health patients which have been previously tested for colistin susceptibility by Etest and have been stored at -70°C. The objective of this study will be to perform antimicrobial susceptibility testing using the colistin broth disk elution method and broth microdilution and compare results of the Etest (currently in use) and colistin broth disk elution method to the standard method- broth microdilution. The colistin broth disk elution method will be evaluated as an alternative method able to produce more accurate results than the Etest method.

The student will be introduced to sterile techniques – including subcultures, freezer banking, and processing of MDR Gram-negative bacterial isolates. The student will also learn how to determine MICs by broth microdilution and colistin broth disk elution methods. Data will be compiled; essential agreement will be determined between methods.

4) ITR Cancer Lab – 1 UNO student

Li Li, MD, PhD and Grace A. Maresh, PhD

Project title: Effect of experimental combination therapies on human kidney cancer in mice with human immune systems

Description:

The student will learn several techniques in order to analyze how kidney cancer is affected by different combinations of therapies in a mouse model system. We have already performed a few of these experiments and need the student to help us do the following: Cut 5 um slices from paraffin blocks of tissues, apply them to microscope slides and stain them with antibodies to important cancer and immune proteins (Immunohistochemistry). These stained slides will be photographed on a microscope and the digital images will be quantitated to find out the results of the experiment. Statistics will be applied to the data.

5) ITR Rheumatology Lab – 1 UNO student

Xin Zhang, MD, PhD

Project title: Identification of the key link between obesity and T cell-mediated autoimmune disease in lupus prone mice

Description:

Systemic lupus erythematosus (SLE or Lupus) is a chronic debilitating autoimmune disease, characterized by inflammation and production of autoantibodies directly against cell components forming immune complex deposits in multiple organs, resulting in skin lesions, arthritis, nephritis, intense fatigue, and potentially life-threatening events. SLE pathogenesis derives from a combination of genetic, environmental, hormonal, and epigenetic factors. Environmental factors, particularly dietary habits have a profound impact on intestinal hemostasis and in general human health. Consumption of high caloric processed food, high fat content food, and low in fiber food has been steadily increasing in our society especially in Louisiana where the incidence of metabolic disease and autoimmune diseases are elevated. Multiple studies have suggested a link between obesity, metabolic syndrome, and autoimmune

diseases including SLE. However, the mechanism linking obesity and lupus remains largely unknown.

The mammalian gut harbors trillions of microorganisms known as the microbiota. Increasing evidence suggests that the host microbiota and immune system interact to maintain tissue homeostasis which plays an important role in metabolic and immunological diseases. The gut microbiota keeps the gut barrier functional and shapes the physiological immune responses in intestine. The host immune system, especially intestinal CD4+ T helper cells protects against harmful gut pathogens but tolerates gut commensals via interaction with IgA secreting plasma cells. Our recent data has shown that high fat diet (HFD)-induced obesity exacerbates lupus symptoms in lupus prone mice, with accumulated T follicular helper (Tfh) cells and active germinal centers, suggesting a unique role of HFD-induced obesity in autoimmune pathogenesis. We hypothesize that high fat diet may collectively induce a compositional and functional shift of gut microbiota, which may induce aberrant immune responses locally and eventually exacerbate systemic autoimmune disease. Our objective of this project is to identify the differences of local (intestine) and systemic (skin, spleen, blood) immune cell profile between regular diet and high fat diet lupus prone mice group. Our long-term goal is to investigate the role of microbiota in obesity, autoimmunity, and inflammation in SLE patient.

Expected outcomes: Student will work on the tissue blocks collected from regular diet and high fat diet lupus prone mice. After completing CURE program, student will gain biological research experimental skills including tissue embedding, slides cutting, hematoxylin and eosin (H&E) staining, immunohistochemical and immunofluorescent staining, and deconvolution microscopy, and digital image analysis. Student will also learn scientific skills including graph making and statistical analysis in this project. He/she will present result in our weekly lab meeting, monthly Rheumatology Department meeting, and have opportunities to gain authorship in our abstract/poster of their research such as Ochsner Research Day.