OCHSNER- UNO COLLABORATIVE UNDERGRADUATE RESEARCH EXPERIENCE (CURE) - 2020 Projects

1) CURE student proposal from Dr. Ifeanyi Iwuchukwu and Dr. Doan Nguyen

Institute of Translational Research
Neuroscience Laboratory
(1-2 students)

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.

Project 2. miRNA-mRNA inflammatory transcriptional regulatory network associated the NF-kB signaling cascade in post-ICH late seizure.
The student will carry out total RNA extraction of blood cells from Paxgene RNA blood tube. Real-time RT-PCR analysis of COMMD6 and APOBEC2, regulators of NF-kB activation, which were found downregulated by RNA-Seq analysis of blood cells in post-ICH late seizure. Plasma will be used for analysis of proinflammatory cytokines and chemokines such as IL-8, IL-1B, and TNF-alpha.

2) CURE student proposal from Dr. George Pankey and Deborah Ashcraft

Institute of Translational Research, Ochsner Health System
Infectious Disease Laboratory
(1 student)

Title: **In Vitro Interaction of Cranberry Proanthocyanidin and Doxycycline Against Escherichia Coli**

Description of Project:
E. coli is the most common pathogen causing urinary tract infections (UTIs). The emergence and spread of multidrug-resistant *E. coli* is a problem worldwide. Resistance to the most frequently prescribed antibiotics used to treat UTIs is occurring, which can make treatment of multidrug-resistant *E. coli* difficult. There is a lack of

development of new antibiotics, so alternative therapies are needed. Combination therapy with antibiotics or even non-antibiotic substances may be used as an attempt to overcome bacterial resistance.

Recently, Maisuria et al. found that cranberry proanthocyanidin (cPAC), when combined with tetracycline, was able to potentiate the activity of tetracycline against *E. coli* and *Pseudomonas aeruginosa*. The American cranberry (*Vaccinium macrocarpon* L.) fruit has long been reported as a remedy for UTIs. In this study, cPAC was found to increase the outer membrane permeability of bacterial cells and inhibit multidrug-resistance efflux pumps. These results suggest that cPAC has the potential to decrease antibiotic resistance and prolong effectiveness of current antibiotics.

The objective of this *in vitro* pilot study will be to combine cPAC with doxycycline (a tetracycline antibiotic) and test against *E. coli*, including multidrug-resistant strains. Minimal inhibitory concentrations (MICs) will be determined for cPAC and doxycycline by a standard broth microdilution method. We will also perform synergy testing using a checkerboard broth microdilution method to evaluate the combination of cPAC and doxycycline at various concentrations to determine any synergistic, indifference, or antagonistic effects.

The student will be introduced to sterile techniques- including normal collection, subcultures, freezer storage, and processing of bacterial isolates. The student will learn how to determine MICs by broth microdilution and perform synergy testing by the checkerboard method.

3) CURE student proposal from Dr. Juan Q. Carlos Velez and Frank Abbruscato

Ochsner Institute of Translational Research
Nephrology Laboratory
(1 student)

Title: **The Role of Aminopeptidase A in the Progression of Chronic Kidney Disease and the Loss of Glomerular Podocytes**

Description of Project:
Aminopeptidase A (APA) is expressed in glomerular podocytes and tubular epithelia and metabolizes angiotensin II (Ang II), a peptide known to promote glomerulosclerosis. Kidney injury is observed in association with increased intrarenal Ang II accumulation in the absence of APA, suggesting a protective metabolizing role of APA in Ang II-mediated glomerular diseases. Our laboratory uses immuno-fluorescent microscopy to study kidney localization of aminopeptidase A throughout the progression of the disease.
In this project, the student will gain a detailed understanding of kidney structure and glomerular function as well as the disease processes involved in chronic glomerular diseases, such as in diabetic nephropathy of focal segmental glomerulosclerosis (FSGS). Moreover, the student will learn about the crucial role of podocytes in normal glomerular physiology, and how injury to them results in loss of kidney function and progression to kidney failure. The student will also be learning about antibodies, how they are made, how they function, and how they are used in different types of immunoassays and methodologies to conduct medical research.

4) CURE student proposal from Dr. Li Li and Dr. Grace Maresh

Institute of Translational Research
(1 student)

Title: **Analysis of a=Activated T Cell Biomarkers in Humanized Immune System Mice with Colon Cancer or Renal Cell Carcinoma**

Description of Project:
In order to study human colon cancer and renal cell carcinoma in mice, we have started testing a method to give mice human immune cells, which will interact with the cancer cells in a similar way they do in humans. This way, we can treat the mice with various therapeutic drugs and look at the response of the cancer and immune cells. If the human immune cells we have given the mice are interacting with the cancer cells, they will produce activated T cell biomarkers. This is what we would like the CURE student to learn – how to set up an assay to detect these biomarkers and then use it to test our experimental samples. This will tell us whether some therapeutic drugs had effects on the cancer through interaction with the immune cells.

5) CURE student proposal from Dr. Ari Cohen, Dr. Paul Thevenot and Dr. Kelley Nunez

Ochsner Institute of Translational Research
Transplant laboratory

(1 student)

Title: **Immune Inflammatory Biomarkers After Primary Treatment in Early Stage HCC Patients: Associations with Treatment Outcomes**

Description of Project:
Chronic viral infection (Hepatitis B/C) and fatty liver disease caused by chronic alcohol use and/or metabolic disease are the major causes of inflammation in the liver. Chronic liver inflammation, or hepatitis, leads to cirrhosis which is characterized by immune cell infiltration, liver injury, and fibrotic changes ultimately leading to liver failure. The constant cycle of inflammation, liver injury, and repair during cirrhosis dramatically increase the risk of develop liver cancer, or hepatocellular carcinoma (HCC). If diagnosed at an early stage, liver transplantation is the best treatment option for cirrhotic patients with HCC, as transplantation removes not only the cancer but also underlying liver disease which led to HCC. Unfortunately, HCC is a very aggressive tumor which can rapidly metastasize to other tissues if left untreated. Many patients who receive liver transplants for HCC experience tumor recurrence within 3 to 5 years after transplantation. HCC recurs because of tumor cells which escape the primary tumor site, where they can survive undetectable in the circulation or as small micrometastases outside the liver.

Our laboratory uses a combination of clinical data and patient-derived specimens to study how HCC and cirrhosis influence key immune populations which can block immune response directed tumors. Projects available to program students this year are listed below:

Immune inflammatory biomarkers after primary treatment in early stage HCC patients: Associations with treatment outcomes.
Since 2016, our laboratory has enrolled and banked specimens from HCC patients undergoing tumor-directed treatments with chemotherapy or radiotherapy eluting beads as part of an ongoing prospective research study. This data, containing both clinical and experimental research variables, will be used by the student to tabulate

several immune risk indices at defined periods before and after treatments and over the time period they were waitlisted for liver transplantation. These indices will be used to determine which approach is ideal for predicting response to treatment and overall outcome. We will further breakdown the analysis to focus on changes in the biomarker indices for specific treatment pathways.

The immunomodulatory function of bilirubin during T cell activation.
Our lab has identified an imbalance in albumin and bilirubin present in a subgroup of cirrhotic patients. This imbalance has important implications on the immune system in cirrhotics, including how they respond to tumor-directed therapy. Specifically, we have focused on the role of elevated bilirubin (hyperbilirubinemia), and how this condition alters T cell and myeloid cell function in a manner which may negatively influence therapy outcomes. In this study, we will use clinical datasets to highlight these relationships between bilirubin and immune modulations in patients with HCC. We will study how a specific T cell subtypes, change their function as bilirubin levels increase. The student will learn how specific T cell and myeloid cell populations are isolated from peripheral blood as well as several strategies to study T cell behavior in cell culture. Specifically, we will study the influence of increasing concentrations bilirubin on T cell and myeloid behavior as well as the mechanisms by which bilirubin alters these responses.

Treatment induced progression of early stage HCC.
There is currently no clinical approach to assess the aggressiveness of HCC at the time of diagnosis and primary to initial treatment. Approximately ~20% of patients undergoing locoregional therapy rapidly progress following treatment. The goal of this study is to utilize clinical and experimental variables previously collected by our lab to determine what potential factors could be used to identify aggressive tumor biology prior to treatment. We will then use this strategy to look for treatment-induced aggressive tumor response in patients following each treatment in patients who have are undergoing repeated treatments or change in treatments. Finally, we will determine whether aggressive transformation is linked to a specific therapeutic intervention or liver disease etiology.

6) CURE student proposal from Dr. Li Li and Dr. Xin Zhang

Institute of Translational Research
(1 student)

Title: **Effect of Fat-Diet Induced Obesity on Lupus Autoimmunity**

Description of Project:

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of autoantibodies, binding with self-antigens to form immune complexes which deposits in various organs potentially causing life-threatening renal, cardiac, or brain damage.

In a prospective cohort study among 238,130 women in the Nurses' Health Studies, the investigators found an 85% significantly increased risk of SLE among obese compared to normal body mass index (BMI) women in the more recent NHSII cohort, but no association was observed in the earlier NHS cohort. Obesity has been considered as a major factor contributing to the onset and progression of autoimmune diseases including SLE. However, the underlying mechanisms are not clear.

Accumulative evidence has shown that T cell subsets play an important role in SLE and other autoimmune diseases. *Under physiological conditions, T follicular helper (Tfh) cell*, a $CD4^+$ T cell subset, predominantly located in lymphoid follicles, produces cytokine IL-21 to regulate B cell survival and antibody production in germinal

centers. *T regulatory (Treg) cell* is an inhibitory T cell subset secreting IL-10 and TGF-β. Thus, the balance among these T cell subsets is very crucial in maintaining proper immune status by developing a robust humoral immune response to foreign antigens and self-tolerance by preventing antibody against self-antigens. *Uncontrolled generation of these T cell subsets* could lead to autoimmunity by increasing autoantibody production and inflammation. Understanding the molecular mechanisms of their regulation is of critical significance in T cells-associated host defense, autoimmune diseases, and cancers.

Our preliminary data have demonstrated compelling evidence that circulating Tfh cell was significantly increased in active SLE patients, correlated with their autoantibody titer and inflammation respectively. *Based on our findings, we have formulated a novel hypothesis that the high level insulin in obesity regulated the downstream signals regulating T cell subsets development and mediating higher level of autoantibodies and stronger inflammatory responses, thereby promoting lupus initiation and progression*. To test this hypothesis, we propose to achieve the following specific aims:

Aim 1. To establish a diet-induced obesity animal model and determine if obesity enhances SLE development.
Aim 2. To reveal the T cell component and the mechanism underlying the link between obesity and SLE development.

Under the collaboration with Tulane University and overlooked by Dr. Robert Quinet and Dr. William Davis from Rheumatology Department, we have successfully established a reliable diet-induced obesity SLE mouse model in 2018 and received award from 2020 Southern Regional Meeting.

The student in CURE program will learn to examine the percentage of Tfh cells and Treg cells in the blood and spleen in MRL/Lpr mouse using flow cytometry. The student also will learn pathological skills such as tissue embedding, slides cutting, hematoxylin and eosin staining, immunohistochemical staining, and microscopic skills. The student will apply these skills on the specimens collected from the diet-induced obesity mice and control mice. This study will help us on determining the frequencies of T cell subsets, immune complex deposit in the kidneys, and the link between obesity to their lupus status. We will discuss the results regularly with staff scientist and physicians/fellows.