LSU Health Sciences Center

2011 Medical Student Summer Research Symposium

ABSTRACT BOOK

LVC Louisiana Vaccine Center

Patrick F. Taylor Foundation

National Heart Lung and Blood Institute
People Science Health

2011 Summer Research Internship Program

This program was started to provide research experiences for medical students, undergraduate and high school students in Louisiana. The program directors Dr. Paula Gregory & Dr. Fern Tsien match students with mentors in laboratories or clinics at LSU Health Sciences Center, Tulane University Health Sciences Center or Children's Hospital of New Orleans. The 8-week summer research program allows students to cultivate their interest in pursuing research careers in either basic or clinical sciences. During the program students conduct their own small research project or work on part of an on-going research project.

Drs. Gregory and Tsien would like to extend their special appreciation to all mentors and poster session judges who helped make the Summer Research Internship a success! Their assistance with this project affords each student to be a part of a bigger, ongoing research project. The Directors would also like to thank supporters of this program: LSU Health Sciences Center, Louisiana Vaccine Center, Patrick F. Taylor Foundation, National Heart, Lung & Blood Institute and Tulane University School of Medicine.

Medical Student Research Symposium 2011
Schedule of Presenters

8:30	**Opening Remarks by Drs. Gregory & Tsien**		
	Medical Student	**Medical School**	**Mentor**
8:40	Ferdous Kadri	LSU	Peruzzi
8:50	Minmin Luo	LSU	Kim
9:00	Brendan Burn	LSU	Claycomb
9:10	Benjamin Morehead	LSU	Lazartigues/Xia
9:20	Stephen Ford, Jr.	LSU	Harrison-Bernard
9:30	Douglas James	Tulane	Ashan
9:40	Carol Faulk	LSU	Richter/Hutcherson
9:50	Marco Rajo	Tulane	Prieto
10:00	Elise Boos	LSU	Molina
10:10	Break	Break	Break
10:20	Scott Melton	LSU	Hagensee
10:30	Tony Wang	Tulane	Beckman
10:40	Charles Murphy	Tulane	Alvarez
10:50	AnhThao Nguyen	LSU	Del Valle
11:00	Valerie McMurtry	LSU	Ferris
11:10	Naoki Murai	LSU	McDonough
11:20	Jennifer Bouso	LSU	Sturtevant
11:30	Amanda Henne	LSU	Bazan/Calandria
11:40	Crystal Leach	LSU	Moreno-Walton
11:50	Kyle Pfefferle	LSU	Lukiw

Elise W. Boos

Medical Student
LSU School of Medicine, New Orleans, L.A.

Mentor:
Dr. Patricia Molina, MD, PhD

Professor and Head Department of Physiology LSUHSC, Director of Alcohol and Drug Abuse Center of Excellence

Impact of Chronic Δ-9-Tetrahydrocannabinol (THC) Administration on Vaginal Markers of Inflammation and Viral Shedding

Previous studies from our laboratory have demonstrated that Δ^9-THC administration decreases simian immunodeficiency virus (SIV) disease progression, increases survival and decreases viral replication without significantly altering the SIV-mediated changes in lymphocyte phenotype, or decline in CD4+/CD8+ lymphocyte ratio in male rhesus macaques. Moreover, our studies showed significant suppression of tissue inflammation and viral replication. Whether similar protective effects are achieved in female SIV-infected macaques is not known. The cervicovaginal compartment is the major route of heterosexual HIV acquisition and transmission and the primary site of HIV transmission from mother to child. Thus, understanding the effects of Δ^9-THC on viral shedding in the cervicovaginal compartment in rhesus macaques is relevant as strategies to diminish risk of transmission are developed. We examined the impact of chronic Δ^9-THC administration (0.32 mg/kg, 2x daily) on levels of cytokines and chemokines and on viral shedding in the vaginal compartment of female rhesus macaques inoculated intravenously with SIV (SIVmac251; 100 $TCID_{50}$/ml, iv) 28 days into chronic Δ^9-THC therapy. Estrus cycle, vaginal pH and cell counts were obtained during the first 3 months post infection. Blood samples were obtained for progesterone and viral load determinations. Cervicovaginal secretions were collected using Merocel sponges and cotton swabs. Following extraction optimization, cytokines and chemokines were extracted from Merocel sponges and the supernatant collect analyzed with Luminex Multi-plex. Cytokine concentrations were normalized to protein levels measured colorimetrically. Viral load was determined by RT-PCR in fluids obtained with cotton swabs. Results obtained will be correlated with circulating progesterone and plasma viral loads. We predict our findings will inform on whether Δ^9-THC exerts local immunomodulatory effects in the cervicovaginal compartment which may decrease HIV replication and shedding. Supported by DA020419 and DA030053 & LSUHSC Summer Research Program.

Jennifer M Bouso

Medical Student, L2
LSUHSC Medical School, New Orleans, LA

Mentor: Dr. Joy Sturtevant
LSUHSC Department of Microbiology, Immunology, and Parasitology

Virulence-attenuated mutants of *Candida albicans* and their modulation of in-vitro cytokine response

Candida albicans is an opportunistic yeast that lives commensally as normal flora in the general population but poses an imminent threat to immunocompromised individuals. Over 80% of HIV-positive patients suffer from the mucosal infection, "thrush," which has a very high morbidity. Under certain environmental conditions, *C. albicans* can become systemic, causing a rapid disseminated infection. The mortality rate of systemic candidiasis can be as high as 40-60% due to diagnostic difficulties and limited options of drug therapies. While the host-pathogen interaction that allows for systemic infection is still unclear, much progress has been made to identify virulence and survival factors of *Candida*. Recently, studies have shown relationships between certain virulence factors and the innate immune response of macrophages and polymorphonuclear cells (PMNs), as well as other immune cells. Thus, it is plausible that some virulence factors alter or exploit certain host immune response mechanisms as a means of survival or invasion. We investigated altered immune responses of murine-model RAW 264.7 macrophage-like cells when incubated with the wildtype SC5314 (*C. albicans* clinical isolate) and mutants with defective abilities to adapt to changes in environment and varying degrees of attenuated virulence. We measured cytokine production of TNF-α, a pro-inflammatory cytokine associated with a beneficial TH1 immune response and IL-10, an anti-inflammatory cytokine associated with suppression of a TH1 response and increased virulence of the organism. We found that time of exposure, concentration of yeast to macrophage, and the strain of *Candida* influences the degree of *in-vitro* TNF-α expression, which may be involved with virulence of the organism. Surprisingly, we found no significant increase or decrease in IL-10 production elicited by our strains of *Candida*.

Brendan Ross Burn

MD/PhD Student

Louisiana State University School of Medicine, New Orleans, LA.

Mentor: Dr. William Claycomb

Department of Biochemistry & Molecular Biology

Shox2 as a marker to study temporal development of the SAN in embryoid bodies.

The sinoatrial node (SAN) serves as the primary pacemaker of the heart. Developmental defects of the SAN can cause symptoms ranging from arrhythmias to sudden cardiac death and represent a major cause of morbidity and mortality world-wide. The SAN is the first functional component of the heart to develop, however little is known about its induction and specification.

The SHOX (short stature homeobox) gene family of transcriptional regulators consists of two closely related members in humans, SHOX and SHOX2. Mice possess a Shox2 homologue, 99% similar to SHOX2 at the amino acid level. Within the mouse heart primordia, Shox2 is expressed in a highly restricted pattern within the sinus venosus myocardium (including the future SAN and venous valves). $Shox2^{-/-}$ mice exhibit embryonic lethality between E11.5-13.5 resulting from bradycardia and severe hypoplasia of the SAN and venous valves. In developing zebrafish embryos, induced Shox2 deficiencies lead to decreased pacemaking function. Shox2 has been shown to participate in an intricate signaling network involving proteins important for heart development, including Bmp4, Tbx5, Nkx2-5, Tbx3, Hcn4, Nppa and Cx43. Together, these data suggests an important function of Shox2 in the developing SAN.

Murine and human embryonic stem (ES) cells cultured as three-dimensional aggregates termed embryoid bodies (EBs) demonstrate the formation of self-organized structures and systems that recapitulate spatial and temporal aspects of early development. The Claycomb laboratory has shown that, in differentiating mESCs grown as EBs, clusters of functional cardiac pacemaking "nodes" as well as conducting cells can be identified *in vitro*. Based on these observations, we hypothesized that the temporal development of the SAN is recapitulated in EBs. To test this hypothesis, we compared EBs generated from wild-type and $Shox2^{lacZ/lacZ}$ (functional knockout) murine ESC cell lines. EBs were analyzed at days 4, 6, 8, and 10 of differentiation. RT-PCR analysis of Shox2, Bmp4, Tbx5, Nkx2-5, Tbx3, Hcn4, Nppa and Cx43 mRNA expression was performed. Immunohistochemical techniques were used to study protein expression of both Shox2 and HCN4, a known marker of the SAN. These studies were used to elucidate the earliest time point of SAN development in this *in vitro* mouse model of embryonic development.

Carol H Faulk

Medical Student
LSUHSC, New Orleans, LA

Mentors: EO Richter and LE Hutcherson
Neurosurgery Department

Effect of experimental methods on a Rhesus monkey's anxiety behaviors

In the United States, more than 12% of men and 20% of women will have an episode of Major Depressive Disorder (MDD) during their lifetime. MDD is characterized by a variety of symptoms including feelings of sorrow or irritability and changes in eating and sleeping patterns. More than half of patients with MDD also have some kind of anxiety disorder. Despite the prevalence of MDD, the pathological mechanism of this disease is not well established. Previous studies have shown that hypoactivity of the dorsolateral prefrontal cortex (DLPFC) is implicated in the mechanism of MDD, specifically with the development of negative mood states.

In this study, high frequency stimulation (HFS) was used to inactivate the DLPFC in a rhesus monkey and the resulting behaviors observed. To activate the HFS, the monkey's cage was collapsed potentially increasing stress and anxiety levels. To account for the possible increase in anxiety, video recorded the hour after the start of stimulation was compared to video recorded two hours after the start of stimulation. The monkey's behavior was noted every second and scored based on 16 different categories. While results are preliminary, collapsing the cage caused a change in anxiety behaviors in the Rhesus monkey. Accounting for the effects of experimental methods prevents the inclusion of skewed data in the final figures. The reliable data will then help us to further understand the abnormal brain circuitry of MDD.

Stephen M. Ford, Jr.

Medical Student
LSU Health Sciences Center School of Medicine; New Orleans, Louisiana

Mentor: Dr. Lisa M. Harrison-Bernard, Department of Physiology
LSU Health Sciences Center – New Orleans

The goal of the research project is to test the hypothesis that upregulation of chymase in the diabetic kidneys is responsible for ACE-independent formation of Angiotensin II.

Under normal physiological conditions, angiotensin converting enzyme (ACE) is the key enzyme responsible for converting the decapeptide angiotensin I into the octapeptide angiotensin II. Under pathophysiological conditions, mouse mast cell protease-4 (mMCP-4), or chymase, is an enzyme formed by and released from mast cells which cleaves angiotensin I into angiotensin II. In the kidneys of Type 2 Diabetic mice, ACE levels are downregulated; however, angiotensin II levels are normal. With decreased ACE levels but normal angiotensin II levels, there must be upregulation of another enzyme resulting in the formation of angiotensin II. Working with Dr. Lisa Harrison-Bernard during the summer, we tested the hypothesis that upregulation of vascular chymase mRNA expression and enzyme activity in the diabetic kidneys is responsible for the formation of angiotensin II.

Isolation of renal vasculature from both diabetic and control mice was followed by qRT-PCR for the specific purpose of quantifying the mRNA levels of chymase localized to these vessels from both groups. In comparing the levels of chymase expressed in the kidneys from the two groups, a statistically significant difference in the expression of chymase factored for beta-actin mRNA expression was detected, with chymase being expressed in vessels from diabetic kidneys ($n = 7$) at five times the expression level ($5.1 +/- 1.38$) in vessels from control kidneys ($n = 7$).

In continuing our work with the expression of chymase within the renal vasculature of diabetic mice, the next step is to isolate the renal vasculature from both diabetic and control mice for the purpose of performing a chymase activity assay. mRNA levels indicate that chymase is significantly upregulated in the diabetic renal vasculature, but the mRNA levels only indicate that DNA transcription is occurring under these conditions. With the next step of performing an enzyme activity assay, we expect to see the translated protein expression of chymase to be present in diabetic renal vasculature at a quantity that is significantly greater than the translated protein levels found in normal, control renal vasculature. In conclusion, these results support the hypothesis that upregulation of chymase causes the ACE-independent formation of angiotensin II within the renal vasculature of the diabetic kidneys.

Amanda Cristine Henne

2nd year Medical Student

Louisiana State University- School of Medicine, New Orleans, LA

Under the mentoring of Dr. Nicolas G. Bazan and Dr. Jorgelina Calandria

Novel regulatory mechanism of the inflammatory response mediated by the messenger NPD1 targeting transcription of Wnt5a

Neurodegenerative diseases share common pathophysiological mechanisms that include enhanced inflammatory signaling and apoptosis. The retinal pigment epithelial cells (RPE) are necessary for photoreceptor cell integrity and vision. In Age Related Macular Degeneration RPE cell damage is involved in the initiation and progression of both forms of the disease: the wet, proliferative and in the dry, atrophic forms. RPE support the daily renewal of photoreceptor membranes rich in DHA (docosahexaenoic acid) and is an integral part of the barrier between the choroid and photoreceptors. DHA, an omega-3 essential fatty acid, is highly concentrated and avidly retained in both the brain and retina, mainly in photoreceptors. DHA is converted into Neuroprotectin D1(NPD1) through enzymatic oxygenation carried out by 15-LOX-1 in the RPE. NPD1 has the ability to promote survival by blocking pro-inflammatory and pro-apoptotic signaling that occurs in stressed RPE cells through the regulation of genes expression.

Wnt5a is a secretory peptide that has been shown to have pro-inflammatory properties. We tested the hypothesis that the messenger NPD1modulate enhanced inflammatory responses in the RPE by restricting Wnt5a activity. We found that Wnt5a activates the COX2 promoter, and along with oxidative stress, potentiates apoptosis in RPE cells. PPARγ is a negative regulator of Wnt5a transcription. To test the effects of Wnt5a on RPE cells, as well as the ability of NPD1 to counteract this pro-inflammatory signal, cells were treated with oxidative stress in either the presence or absence of NPD1. The addition of NPD1 attenuated the increase in Wnt5a levels induced by oxidative stress. To assess the role of PPARγ in the NPD1-mediated regulation of Wnt5a, human retinal pigment epithelial (hRPE) cells were treated with either troglitazone (TRO), potent activator of PPARγ, GW9662, a potent inhibitor of PPARγ, or neither as a control. We then performed a western blot on the hRPE cells measuring the levels of Wnt5a under the different conditions. We found that TRO diminished the levels of Wnt5a in the presence of oxidative stress and this effect was potentiated by NPD1. On the contrary, GW9662 derepressed the expression of Wnt5a even during resting conditions, causing a high level of Wnt5a in non-stressed cells. Our results uncover that NPD1 is a regulatory inhibitor of Wnt5a at the transcriptional level in stressed hRPE cells and suggests that PPARγ is involved in the regulation of the expression of Wnt5a.

All together this data suggests that NPD1 is a regulator of Wnt5a, and therefore, is a regulator of pro-inflammatory signaling within stressed RPE cells. These events may be important in the initiation and early progression of AMD. Therefore this can be used as a potential treatment for neurodegenerative diseases that involve enhanced inflammation, including AMD.

Douglas E. James

Medical Student
Tulane University, New Orleans, LA

Mentor : Taby Ahsan, PhD
Tulane University
Department of Biomedical Engineering

Characterization of embryoid bodies separated by Percoll density gradient centrifugation

Embryonic stem cells (ESCs) with the potential to differentiate into all cell types are a powerful source for tissue engineering, pharmaceutical testing, and basic science research. However, large numbers of cells will be required in order to make these applications useful. When cultured in suspension, ESCs form aggregates termed embryoid bodies (EBs). EBs recapitulate the early stages of embryogenesis and have the ability to differentiate into all three germ lineages – ectoderm, mesoderm, and endoderm. While EBs can be cultured on a large scale they will have variation in size and morphology. We hypothesized that these heterogeneous populations of EBs would have different densities and density gradient centrifugation with Percoll could provide a useful and inexpensive tool to isolate cells for downstream applications.

We cultured the murine embryonic stem cell line ES-D3 in medium supplemented with leukemia inhibitory factor (LIF). LIF is an IL-6 family cytokine that has been identified as a factor that maintains the pluripotency of ESCs. The ESCs were then trypsinized and replated in non-tissue culture treated Petri dishes in medium without LIF and cultured on an orbital shaker at 40 RPM. These culture conditions have been shown to produce a more homogenous EB population than static culture methods. We found that even using this method, the EB population becomes more heterogeneous with time. We assessed EB heterogeneity by measuring the area and circularity of EBs. The size of an EB has been found to affect its differentiation trajectories and efforts to control EB size in culture is an active area of stem cell research.

Density gradients were made using Percoll. Percoll is a solution of silica colloid particles that are 15-30 nm that are coated with polyvinylpyrrolidone. After culturing EBs for 4, 8, and 12 days we performed density gradient centrifugation with Percoll at densities from 1.040-1.065 g/ml at intervals of 0.005 g/ml. Day 4 EBs were found to mostly have densities between 1.050-1.055 g/ml. The day 8 and day 12 EBs had a population lighter than 1.040 g/ml and what seemed like another population between 1.050-1.055 g/ml. We stacked gradients using the densities 1.055, 1.050, and 1.045 g/ml resolve distinct populations. Analyzing the fractions using qRT-PCR, we found that at day 12, the denser the fraction, the higher its expression of Nanog. Nanog is a marker for pluripotency in ESCs. Further studies will be required to confirm these results and further assess the characteristics of EBs of different densities. Ultimately, sorting of EBs with Percoll will provide an inexpensive method for selection of desired cell types.

Ferdous Kadri, MSc.

Medical Student

Louisiana State University Health Science Center, New Orleans, LA

Mentor: Francesca Peruzzi, PhD.

Louisiana State University Health Science Center

MicroRNA Signature of HIV-Associated Neurological Disorders

HIV-1 can cause Central Nervous System (CNS) dysfunction leading to dementia, motor dysfunction, and memory loss. Diagnosis of HIV-1 associated neurological disorders (HAND), particularly HIV-1 encephalitis (HIVE), presents a real challenge because of a lack of biomarkers which would allow for characterization of disease status and progression. MicroRNAs, or miRNAs, are 18-25 nucleotides noncoding RNAs that silence gene transcription through imperfect base pairing to the 3' untranslated region (UTR) of an mRNA. Emerging data suggest miRNAs as important gene regulators of proliferation, cell signaling, differentiation, and apoptosis. In the CNS there is strong evidence that altered homeostasis caused by inflammation and neurodegeneration potentiates an altered expression of specific miRNAs. In addition, their stability has allowed the detection in blood, urine, tear, amniotic fluid, and cerebrospinal fluid (CSF). This allows for miRNAs to be characterized as exemplary biomarkers in body fluids and may reflect upon the specificity of certain pathologies. Along with the fact that HIV-1 alters the expression of microRNAs, we hypothesize that CSF miRNA profiling of HAND, specifically HIVE, can provide us with miRNA signatures unique to this pathogenesis. We obtained CSF samples from five HIV-positive patients without encephalitis and five HIVE-positive patients; total RNA were extracted and used for reverse-transcription reaction with Universal cDNA Synthesis Kit (Exiqon). Employing a high throughput method, real time polymerase chain reaction (PCR) on miRCURY LNA microRNA 384 well plate that contains specific primer for each miRNA was performed. Fluorescent data was obtained to quantify and calculate the fold change using GenEX software (MultiD). Furthermore, differential expression of common miRNAs was calculated across all HIVE patients to yield a quantifiable up-regulation, down-regulation, or unchanged status as compared to HIV-positive with no encephalitis. miRNAs were also found that were either unique to HIVE samples or HIV-positive samples with no encephalitis. The results are indicative of a strong correlation between specific miRNA signatures in CSF and HIVE. However, analysis of a larger sample population is necessary in order to further support our hypothesis that CSF miRNAs may serve as diagnostic and/or prognostic markers.

Crystal I. Leach

Medical Student

Louisiana State University School of Medicine, New Orleans, LA

Mentor: Lisa Moreno-Walton, MD, MS, MSCR
Associate Professor, Emergency Medicine
Associate Residency Program Director, Emergency Medicine
Louisiana State University Health Sciences Center

Do Parents Know How to Perform Pediatric CPR on Their Infants or Children?

INTRODUCTION: Knowing when and how to administer early cardiopulmonary resuscitation (CPR) can prevent the death of infants and children experiencing out-of-hospital cardiac arrest. Drowning, suffocation, poisoning, smoke inhalation and Sudden Infant Death Syndrome (SIDS) are examples of events that are commonly experienced in the pediatric population which can lead to cardiac arrest. Administration of CPR during the early stages of a cardiac arrest has been shown to decrease both mortality and morbidity in infants and children. A reduction in the time between cardiac arrest and the initiation of CPR results in more positive outcomes in pediatric cardiac arrest victims. Providing pediatric CPR trainings and re-trainings to parents, guardians and expecting parents is a simple and effective way to increase the likelihood that this will happen.

OBJECTIVE: To determine the percentage of parents or guardians who know how to effectively perform CPR on infants or children. The study also seeks to identify the percentage of parents or guardians who believe that, after receiving pediatric CPR training, they would be comfortable performing pediatric CPR on their own infant or child or on someone else's infant or child.

METHODS: The participants in this study were parents and guardians whose infants or children were awaiting treatment in the Pediatric Emergency Department at Our Lady of the Lake Regional Medical Center. A convenience sample was collected from weekday, week night and weekend shifts. All participants anonymously completed a survey questionnaire on pediatric CPR training and performing pediatric CPR on children and infants. At the completion of data collection, we will compute the proportion of participants who know how to perform pediatric CPR and are comfortable with performing pediatric CPR within 95% confidence intervals.

RESULTS: Three hundred parents and guardians were enrolled in the study. Data is being entered onto an excel spread sheet and will be analyzed early next week.

CONCLUSIONS: Will be provided in the oral presentation of this study. We hypothesize that the majority of parents will not have been trained in CPR and will not express comfort in the performance of this skill.

Minmin Luo

Medical Student

Louisiana State University Health Sciences Center School of Medicine, New Orleans, Louisiana

Mentor: Dr. Sunyoung Kim

Department of Biochemistry and Molecular Biology, LSUHSC-New Orleans

OBSERVING THE TRANSITION STATE OF A MOLECULAR MOTOR IN REAL-TIME

Eg5 is a motor protein essential to cell division because it couples ATP hydrolysis to organizing microtubules into a metaphase spindle. Its role in mitosis and its druggability make Eg5 an excellent target for anti-cancer therapy. Although there are many small-molecule allosteric inhibitors of Eg5, there are no transition state inhibitors, because its catalytic transition state has not been determined. The transition state, an unstable chemical intermediate poised between the substrates and products, is central in cellular processes as it lowers the activation energy needed for catalysis. Moreover, chemical analogs that resemble or trap the transition state are powerful inhibitors successfully used in medicine. Examples of transition state inhibitors are the neuraminidase inhibitor Tamiflu, which is a flu medication, and the HIV protease inhibitors Fortovase and Invirase in the treatment of AIDS.

Difference Fourier transformed infrared spectroscopy is useful for monitoring the secondary conformational changes to protein structure over time. Our research applies this methodology to identify residues and structural changes required in the catalytic transition state of the human Eg5 kinesin through real-time monitoring of an *in vitro* ATP hydrolysis reaction. The long term goal of this project is to provide novel information on how protein force and motion are linked to transition state formation and allow for rational drug design targeting this catalytic intermediate. Here, we present results of FTIR using both non-labelled Eg5 and global ^{15}N-labeled Eg5, which isolate secondary conformational changes, as well as the preliminary data for isotopic labeling of specific amino acids, which can be used in FTIR to determine the identity of the residues undergoing dynamic chemical changes during transition state formation.

Valarie E. McMurtry

Medical Student
Louisiana State University Health Science Center, New Orleans, LA

Mentor: Mike Ferris, PhD
Microbiology, Immunology, and Parasitology Department, Children's Hospital

The role of the hospital environment in the bacterial colonization and disease

Necrotizing enterocolitis (NEC) is a common gastrointestinal inflammatory disease in preterm infants. NEC is distinguished from other gastrointestinal diseases by the death of epithelial cells lining the intestine. The exact cause of NEC is unknown, but it is theorized that specific bacterial species that colonize the gut cause inflammation and eventually death of the epithelial cells in the gut. It has been found that a predominance of *Proteobacteria* and/or a limited diversity of bacteria colonizing the intestine can predispose an infant to NEC. When an infant is in the womb, its gut is sterile. Soon after birth, the gut is colonized by bacteria from the environment. Normally, infants that are delivered vaginally have an intestinal microflora that reflects the microflora of the mother's vagina. Infants that spend substantial time in a hospital environment, such as the neonatal intensive care unit (NICU), develop a different microflora and are at higher risk for NEC. To begin to understand which bacteria in a hospital environment might colonize the gut, we sampled various, high-traffic locations in the NICU including light switches, door handles and keyboards. We also samples areas were air born bacteria and dust collects, above light fixtures, and in sink drains where microbial biofilms form. To assess whether microbial species in the NICU environment vary over seasonal intervals, we sampled the same sites in the winter and summer. We extracted DNA from the specimens, used PCR with general bacterial primers to amplify the 16S ribosomal RNA genes and sequenced them to indentify species in the NICU. We found that *Streptococcus* was the most prevalent and abundant bacteria in the summer samples while *Burkholderia* was the most prevalent and abundant winter samples. We found an abundance of halophiles in a carpet located in front of a common sink in the winter, but not in summer. Certain genera such as *Kocuria* and *Corynebacterium* were prevalent in both seasons. Preliminary data suggest that bacterial composition in the NICU changes over a seasonal interval.

Scott J. Melton

Medical Student
Louisiana State University School of Medicine, New Orleans, LA

Mentor: Michael Hagensee, MD, PhD
Louisiana State University Health Sciences Center

Epstein-Barr Viral Infection: A Factor in Human Papillomavirus Related Cervical Dysplasia in HIV$^+$ Women?

Background: Human Papillomavirus (HPV) is an oncovirus that has been associated with 99% of cervical cancers and has a prevalence of 75-80% among sexually active Americans at some point during their lives. While a majority of women are eventually infected with HPV, only 16 per 100,000 individuals actually go on to develop cervical cancer. This low ratio of cervical cancer to HPV infection strongly suggests that another factor is needed to induce tumorigenesis. Epstein-Barr virus (EBV) is another oncovirus that has approximately 95% prevalence in some studied populations. The majority EBV infections are asymptomatic, although EBV has been linked to mononucleosis, Burkitt's lymphoma, nasopharyngeal carcinoma, and some gastric cancers. The purpose of this study is to determine if patients producing antibodies to HPV-16 are also commonly producing antibodies to EBV and to determine if HPV/EBV positive antibody status correlates with a more severe degree of cervical dysplasia seen on histological exam.

Methods: Women who are HIV$^+$ were enrolled into an ongoing longitudinal study investing the role of EBV/HPV co-infection in cervical dysplasia. Patients were seen in clinic approximately every three months, at which time the patient underwent a pelvic exam including Papanicoulaou smear. Blood was drawn for serum analysis with two separate immunoassays, one for HPV-16 antibodies and the other for antibodies to EBV virus capsid antigen (VCA).

Results: A cross section of the data showed that patients who were positive for HPV-16 antibodies presented more often with some form of cervical dysplasia. EBV/HPV antibody production within the dysplasia group was positively associated using Fisher's exact test ($p = 0.029$). This association was not seen in the patients who were negative for dysplasia ($p = 0.183$). Of the patients who presented with dysplasia and were producing HPV-16 antibodies, those that were also producing EBV VCA antibodies showed more severe forms of dysplasia.

Discussion: The data indicate that EBV infection likely does play a role in cervical cancer dynamics due to the association with HPV seen in the dysplasia group and the observation that the EBV+/HBV+ patients show a greater degree of dysplasia. However due to the limited sample size, EBV status cannot be statistically determined to be a predictor of cervical cancer at this time. Further research into the association of these two variables is needed to determine the effect of EBV+/HBV+ co-infection on cervical cancer.

Benjamin Eric Morehead

Medical Student
LSUHSC-NO, New Orleans, LA

Mentors: Dr. Eric Lazartigues, Dr. Huijing Xia:
LSUHSC Department of Pharmacology and Experimental Therapeutics, LSUHSC, New Orleans, LA

Brain ACE2 prevents the development of Hypertension in DOCA salt mice

Angiotensin Converting Enzyme 2 (ACE2) is a relatively newly discovered component of the renin-angiotensin system (RAS) that helps to degrade angiotensin I and angiotensin II to angiotensin 1-7. Current research suggests that increased ACE2 function might be a candidate treatment for hypertension.

To investigate this hypothesis, we generated transgenic mice which overexpressed a human form of ACE2 (hA). hA and non-transgenic (NT) mice underwent treatment to induce hypertension. The first step was a uninephrectomy (sparing adrenal gland). One week later, the mice were implanted with a telemetry probe for continuous blood pressure (BP) monitoring in conscious free moving condition. One week following this, a 3 day baseline blood pressure reading was taken, and the mice received a subcutaneous DOCA- silicone pellet containing 10mg DOCA/10g body weight. At this time, their water was substituted with a 1% NaCl solution. Three weeks after DOCA-salt treatment, the mice were sacrificed, and western blots for ACE and AT1 receptors (AT1R) protein expression, and an enzyme kinetic assay for ACE2 activity were done on isolated brain tissues. Baseline ACE2 activity (AFU/mg/min) was higher in hA mice brain compared to NT (920±7 vs. 598±4, $P<0.05$), while BP, ACE and AT1R expression did not significantly differ from NT vs. hA mice. Three weeks after DOCA-salt treatment, BP (mmHg) was increased in both NT (146±2.5 vs. 104±2.8, $P<0.01$) and hA (131±4 vs. 106±1.5, $P<0.05$) mice, however, the increase in hA was significantly smaller than NT mice (24±7 vs. 42±3, $P<0.05$). While, ACE2 activity was significantly decreased in NT mice (109±9, $P<0.01$), it remained at a normal level in hA mice (536±5) when compared to NT on baseline. In addition, DOCA-salt increased AT1R (+50.02±12.4%, $P<0.05$ vs. baseline) protein expression in NT mice, but not in the hA mice (-5.83±19.5%, $P>0.05$ vs. baseline, $P<0.05$ vs. NT+DOCA), leading us to conclude that maintenance of ACE2 activity in the central nervous system offers some protection against an increase in AT1R due to DOCA treatment. No statistically significant change was observed in our analysis of ACE protein levels between NT and hA mice, and their DOCA treated counterparts. Our data suggest that brain ACE2 is able to counter-balance the RAS component AT1R, thus ameliorating the development of hypertension.

Naoki Murai

Medical Student
LSUHSC, New Orleans, LA

Mentor: Dr. Kathleen McDonough
LSUHSC New Orleans

Potentiation of endotoxin induced cardiac impairment by chronic alcohol consumption is not correlated with altered levels of pro- or anti-inflammatory cytokines.

Ethanol consumption may improve or impair the function of the cardiovascular system depending on the amount of alcohol consumed and the duration of use. Chronic consumption enhances the release of chemokines and cytokines from Kupffer cells and can deplete CD4+ T lymphocytes impairing the adaptive immune response. Hearts were isolated from our mouse model of chronic alcohol consumption; 20% ethanol *ad libidum* for 8-10 weeks. While these hearts produced cardiac work equivalent to hearts from non alcohol consuming mice, they suffered greater performance impairment when exposed to an injurious stimulus. A single bolus intraperitoneal injection of endotoxin was used as the injurious stimulus. The aim of this study was to elucidate the mechanism(s) for potentiation of endotoxin induced cardiac performance impairment by chronic alcohol consumption. Candidate mechanisms included induction of pro-inflammatory cytokines TNF-alpha and IL-6. Suppression of anti-inflammatory cytokine IL-10 was considered as well. None of these cytokines were correlated with potentiated endotoxin induced cardiac impairment. Future studies will investigate the level of oxidant stress in hearts from alcohol consuming mice. The potential roles of iNOS and altered Ca++ homeostasis in the exacerbation by chronic alcohol consumption of endotoxin induced cardiac dysfunction will also be considered.

Charles Edward Murphy

MD Candidate class of 2014

Tulane University School of Medicine, New Orleans, LA

Mentor: L. Lee Hamm, MD

Department of Internal Medicine

Tulane University School of Medicine

24 Hour Urine Sodium in Hypertensive and Normotensive Rural Haitians: A Pilot Study

Essential hypertension is a multi-factorial disease that is strongly associated with numerous environmental factors. Although commonly associated with developed countries with high sodium diets, where a large percentage of the population is hypertensive, most of the burden of hypertension related disease is borne by low income, developing countries. For many countries, there is a lack of adequate data concerning essential hypertension. Haiti, for example, is a strong illustration of this phenomenon. Here we present a pilot study on 24 hour urine sodium content in rural Haiti. 25 hypertensive and 12 normotensive residents from the rural community of Jacsonville, on the central plateau of Haiti, submitted 24 hour urine samples. Total 24 hour urine volumes were recorded and 50ml samples were collected for analysis of sodium, potassium, osmolality, and creatinine. Blood pressure, height, weight, and BMI were collected from each subject. Buccal mucosa swabs for DNA analysis were collected from each subject with a standard collection kit (Isohelix SK-1 kit with Dri-Capsules). In addition, we conducted a retrospective chart review of 500 medical records on the residents of Jacsonville to determine the incidence of essential hypertension in the community. Prelimary results indicate the incidence of essential hypertension is 31% with an average BMI. in Jacsonville, Haiti. This is unexpectedly high given the low rates of common risk factors such as obesity. The hypertensive individuals in the pilot study appear to have significantly lower BMI, compared to normotensives although neither group was outside of the normal BMI range (19.8 vs. 24.4). Pending urine analysis data and planned DNA analysis should help clarify the possible contributors to unexpectedly high rate of essential hypertension within this community

AnhThao Thi Nguyen

Medical Student

Louisiana State University School Of Medicine, New Orleans, LA

Mentor:

Dr. Luis Del Valle

Louisiana State University Health Sciences Center

Association between JC Virus and Colon Cancer

Background: JC Virus is a human neurotropic virus which infects greater than 805 of the adult population world-wide. The primary infection is sub-clinical and occurs in early childhood, after which the virus remains in latent state in lymphocytes and kidney epithelial cells. Reactivation under immunosuppressive conditions results in the fatal demyelinating disease Progressive Multifocal Leukoencephalopathy. Under other conditions, expression of the early viral protein T-Antigen results in the deregulation of key cell cycle regulator proteins, such as p53 and pRb and malignant transformation. Reports of enduring viability of JCV in acidic environment of sewage samples suggest a potential oral fecal transmission route for JCV. In addition, several reports have demonstrated the presence of viral genomic sequences in the upper and lower digestive tracts. Base on these observations, in the present study, we investigate the association between JC Virus and colon cancer.

Materials and Methods: We collected 48 paraffin embedded samples from patients with colon cancer and we perfomed immunohistochemical experiments in order to detect the presence of the viral oncoportein T-Antigen. In addition, normal and cancer samples were compared for levels of expression of host cell cycle regulator proteins, p53, Rb, β-catenin to examine if there s a correlation with the presenc of JCV and the expression of T-Antigen.

Results: Thus far only one sample has been positive for the detection of T-Antigen. However, p53 is four times more positive and Rb is two times more intense in cancer samples than normal samples. B-catenin expression in the cytoplasm is approximately equal between cancer and normal samples. Interestingly, nuclear expression of B-catenin is detected only in cancer samples.

Future directions: We will re-label our samples for T-antigen with a higher antigen-retrieval temperature and primary antibody incubation at exactly 25 Degree Celsius. If positive for T-antigen, we will proceed with PCR for JCV DNA, double labeling for host and viral proteins in order to demonstrate the binding of these proteins. In addition, we will examine the effects of JCV on DNA integrity and we will examine is T-Antigen in capable of altering the IGF-1/IRS pathway, important for faithful DNA repair.

Kyle P. Pfefferle

Medical Student
LSU Neuroscience Center, Louisiana State University Health Sciences Center

Mentor: Dr. Walter J. Lukiw
LSU Neuroscience Center, Louisiana State University Health Sciences Center

Effects of neuroprotectin D1 (NPD1) on TNFα + Aβ42 peptide-stressed human astroglial (HAG) cells in primary culture; induced changes in micro RNA (miRNA) complexity

Brain tissues consist of a highly integrated network of neuronal signaling and astroglial support cells. Changes to this homeostatic network are apparent in Alzheimer's disease (AD) and are thought to be in part due to the elevated presence and effects of pro-inflammatory cytokines and amyloid-beta (Aβ) peptides. In these studies we have analyzed the pro-inflammatory effects of tumor necrosis factor alpha (TNFα) and Aβ42 peptides on gene expression in human astroglial (HAG) cells in primary culture. We have also analyzed the effects of the docosahexaenoic-acid (DHA) derived neuroprotectin D1 (NPD1) on the quenching of pathogenic signals generated. Our end-points included quantitation of the markers vimentin and glial fibrillary acidic protein (GFAP) for glial cell proliferation and COX-2 as an inflammatory marker in HAG cells. In addition, using micro-RNA (miRNA) array analysis we assayed for consistent changes in miRNA speciation and complexity during the course of TNFα, Aβ42 peptide and NPD1 treatment. Our results indicate specific up-regulation of the inflammation-associated miRNA-125b and miRNA-146a. Overall these results suggest that both miRNA-125b and miRNA-146a contribute to pro-inflammatory signaling in HAG cells, an effect that is quenched in part using NPD1. Anti-micro RNA strategies may be clinically useful in the treatment of astroglial inflammatory and proliferative disease.

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Marco A. Rajo

Medical Student
Tulane University School of Medicine, New Orleans, LA

Dr. Minolfa Prieto, MD PhD
School of Medicine, Department of Physiology and Hypertension and Renal Center, Tulane University

Prorenin Receptor (PRR) expression in the collecting duct in type 2 diabetic db/db knockout mice during angiotensin (ANG) II dependent hypertension

Recently, a new component of the renin-angiotensin system (RAS) has been described, the prorenin/renin receptor (PRR), which by binding renin and pro-renin enhances their activity, thereby contributing to renal injury. The PRR expression is increased in the collecting duct (CD) of ANG-II hypertensive rats and mice. In diabetes, the major source of pro-renin is the CD. However, it has not been determined if PRR is upregulated in the CD during diabetes. In the present study, we aimed to determine the gene expression of PRR in the CD of db/db knockout (db/db) type-2 diabetic mice during chronic ANG II infusion and treatment with insulin. The mRNA and protein PRR levels were examined by Western blot and qRT-PCR in the renal inner medulla of kidneys from male (30g) wild-type (n=5), db/db (n=6), db/db-ANG II infused (1000 ng/min, via SC minipump for four weeks; n=4-5), and db/db-ANG II infused plus insulin (n=4-5) mice. Data suggest that there is an increase in PRR gene mRNA and protein expression in the db/db diabetic mice, which is exacerbated by ANG-II infusions, but ameliorated by insulin treatment. Augmentation of PRR in the CD during diabetes and even further when it is associated to Ang II dependent hypertension may contribute to the exacerbation of renal injury during these conditions.

Tony L. Wang

Medical Student

Tulane University School of Medicine, New Orleans, Louisiana

Mentor: Dr. Barbara S. Beckman

Tulane University School of Medicine

Research Statement

With 1 and 5 year survival rates of 25% and 6%, pancreatic carcinoma has the worst prognosis of any major malignancy. Additionally, it has been shown to be one of the most drug-resistant cancers, with limited responsiveness to most current chemotherapeutic treatments. As a result, complete remission from pancreatic cancer is still rare.

Ceramide, a sphingolipid component of cell membranes, has been shown to play an important role in a variety of cellular functions including apoptosis, cell growth arrest, and differentiation. Recent studies suggest that various extracellular stimuli regulate the relative ratio of ceramide to sphingosine-1-phosphate (S-1-P). Cell stressors can elevate the activity of the enzyme, sphingosine kinase, which increases the amount of S-1-P relative to the amount of ceramide. The increase in intracellular S-1-P to ceramide ratio promotes cell survival and proliferation. On the other hand, an increase in intracellular ceramide levels has been shown to mediate apoptosis and growth arrest in response to TNF-α and certain chemotherapeutic agents. Therefore, targeting this pathway with synthetic ceramide analogs provides a promising therapeutic target in the treatment of various forms of carcinomas.

To this end, our lab has tested a variety of ceramide analogs *in vitro* using various breast and ovarian carcinoma cell lines. Among all the ceramide analogs previously tested, we found that analog 406, *N*-((*S*, 4*E*, 6*E*)-1-hydroxy-3-oxoheptadeca-4, 6-dien-2-yl)-2-phenylacetamide, was the most effective analog in breast and ovarian cancer cells. The present study sought to investigate the *in vitro* efficacy of analog 406 in the pancreatic ductal adenocarcinoma cell line PANC-1 using the MTT viability assay and the clonogenic survival assay. Analog 406 was found to have an IC_{50} value of 8.619 μM in the viability assay and 0.449 μM in the clonogenic survival assay. In comparison, gemcitabine, the most common chemotherapeutic used for pancreatic cancer, has been shown to have an IC_{50} value of 58.24 μM in the MTT viability assay. These results suggest that ceramide analogs may serve as potential chemotherapeutic agents in the treatment of pancreatic cancer.