TEXAS A&M UNIVERSITY

College of Medicine

SUMMER RESEARCH PROGRAM

July 30th, 2021

RESEARCH DAY

Medical Research and Education Building II

Bryan, Texas

SCHEDULE OF EVENTS

July 30th, 2021

9:00AM-12:00PM	Poster Viewing & Judging MREB II Lobby
12:00PM-12:30PM	Lunch MREB II 1403
12:30PM-1:30PM	SRP Keynote Speaker "Vaccine Development: Bench to Bedside" Ramesh Koukuntla, PhD Head of Process Development FUJIFILM Diosynth Biotechnologies MREB II 1403
1:30PM-2:00PM	Presentation of Certificates Brett Mitchell, PhD, FAHA Summer Research Program Director MREB II 1403
2:00PM	Group Picture & Adjourn

CONGRATULATIONS!

Keynote Speaker

Dr. Ramesh Koukuntla
Head of Process Development
FUJIFILM Diosynth Biotechnologies

Title:
Vaccine Development: Bench to Bedside

Ramesh Koukuntla, PhD, currently the Head of Process Development (PD) at FUJIFILM Diosynth Biotechnologies, College Station, leads a large group of Scientists and Engineers in the Upstream, Downstream, Analytical Development, and Process Science functions. Ramesh also leads the Science and Innovation projects for FUJIFILM Diosynth Gene Therapy related programs. Before heading the PD group, Ramesh had a brief stint as the Director, Program Design, Gene Therapy at FUJIFILM Diosynth.

Previously, as a Senior Scientist at Boehringer Ingelheim Animal Health, Ramesh's primary research focus was on the development of Equine Herpes virus and Canine adenovirus based recombinant vaccines against multiple pathogens (from Rabies to FMDV) for use in several animal species including dogs, cats, pigs, and horses.

Ramesh has also worked at NewLink Genetics Corp. and Bioprotection Systems Corp. in Iowa, where he led the development of adjuvanted and virus-like particle based vaccines against emerging and re-emerging hemorrhagic fever viruses and infectious diseases – specifically Rift Valley Fever and Influenza. After completing his Masters in Molecular and Human Genetics from Banaras Hindu University, India, Ramesh graduated with a Ph.D. in Genetics from Iowa State University. Since then, his research and professional focus has been on the development of recombinant viral vectors for use in gene therapy applications and as recombinant vaccines.

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IMPACT OF ADJUVANTS ON LYMPH TRANSPORT

Joseline Alvarez, Wei Wang, David Zawieja
Dept. of Medical Physiology
Texas A&M College of Medicine
Bryan, TX

Adjuvants are substances in vaccines that enhance the body's immune response to an antigen and aid to accomplish more effective adaptive immunity. Yet, the underlying mechanisms adjuvants use to achieve effectiveness remain unknown. Evidence suggests that lymphatic transport may play a crucial role. Because of their characteristics, the vaccine elements must be transported from the injection site to, through and from the nodes via lymphatics. Hence, we hypothesized that adjuvants significantly alter lymphatics connected to and within the node leading to more effective transport and immune responses. We measured lymph transport by examining; the pumping/contractility of isolated, rat mesenteric prenodal lymphatics and the nodal vascular structures in response to adjuvants. We treated the isolated lymphatics with 3 different classes of adjuvants: Adda-vax, CpG ODN, and Alum at 4 transmural pressures. We recorded lymphatic diameters and contraction frequency at each pressure. Videos of the experiments were used to analyze lymphatic contractions and track lymphatic diameters. From the diameter measures, we determined lymphatic pump function and vessel contractility and used statistical analysis to determine the effects of adjuvants on lymphatic pumping/contractility. To begin to evaluate how the adjuvant may affect lymph transport within nodes, we analyzed the nodal blood and lymph vascular structures using immunohistochemistry with and without adjuvant treatments. These data indicate that adjuvants altered delivery of lymph by decreasing both tonic and phasic lymphatic contractions, decreasing lymph pump frequency and fractional flow. We effectively imaged the nodal lymphatic network architecture using Lyve-1 confocal microscopic immunofluorescence histochemistry.

JA was supported by a Fellowship from NIH R25 DK126642.

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DI-TYROSINE BOND MUTATIONS IN ULTRABITHORAX AFFECT THE FORMATION AND FLUORESCENCE OF BIOPOLYMERIC FIBERS

Darius Caldwell, Amanda Jons, Brandon Look-Fong, Sarah Bondos
Dept. of Molecular & Cellular Medicine
Texas A&M College of Medicine
Bryan, TX

Ultrabithorax (Ubx) is a Hox transcription factor that contributes to the development of Drosophila melanogaster *in vivo*. *In vitro*, Ubx self-assembles in mild buffer at the air-water interface. These strong and elastic biomaterials could have a variety of applications in medicine. Techniques that would allow us to better understand (and enhance) materials assembly require materials assembly in extremely low salt solutions. These fibers are more blue fluorescent, indicating a higher number of the di-tyrosine bonds per unit volume. The purpose of this experiment is to compare di-tyrosine bond formation in fibers formed by Ubx and Ubx tyrosine mutants to distinguish between possible sources of additional bonds. We changed the tyrosines at positions 4,12, and 240 to serines, which will not affect the structure of materials, but will prevent formation of known di-tyrosine bonds. We hypothesize that mutant forms of Ubx will fluoresce less than plain Ubx because the di-tyrosine bond interactions are fewer in these mutant forms. The intensity of blue fluorescence in fibers composed of Ubx or its mutants were measured via confocal microscopy. The results of the experiment indicate high protein concentration increases the probability of random interactions forming between tyrosine residues. In addition, mutant forms of Ubx can form more random tyrosine interactions in comparison to plain Ubx at low salt concentrations. Shorter incubation periods in the buffer solution allow random fast-forming bonds to stunt specific interactions between tyrosines. In conclusion new, unidentified, interactions between tyrosine residues are formed in protocols that differ from ours.

This work was supported NIH 2R01 GM099827-06 to SB. DC was supported by a fellowship from NIH R25 DK126642.

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GENETICALLY AUGMENTED RENAL LYMPHANGIOGENESIS ALTERS THE IMMUNE RESPONSE FOLLOWING ACUTE KIDNEY INJURY

Naomi Dillard, Gaurav Baranwal, Joseph Rutkowski
Dept. of Medical Physiology
Texas A&M College of Medicine
Bryan, TX

Acute Kidney Injury (AKI) is characterized by a sudden decline in renal function and studies have shown that there is an increased risk for progression towards chronic kidney disease (CKD). Various renal immune cell populations following an AKI play an important role in its pathophysiology and the potential for CKD progression. In AKI, Th1 and Th17 subpopulation of T-cells have been identified as detrimental toward recovery resulting in CKD while increasing the number of Tregs may prevent progressive injury. Lymphatic vessels, and inflammation-associated lymphangiogenesis, help to restore homeostasis following tissue injury. In the present study we hypothesized that increasing renal lymphangiogenesis would help to reduce pro-inflammatory immune cell numbers in the kidney and thus improve the likelihood of recovery following an AKI. Our lab has recently characterized transgenic mice that overexpress the potential lymphangiogenic signal VEGF-D only in the kidney upon doxycycline administration. These conditional “KidVD” mice exhibit marked lymphangiogenesis throughout the kidney. To test our hypothesis, we utilized KidVD mice in two models of kidney injury: cisplatin nephrotoxicity and isolated glomerular injury. We first induced lymphangiogenesis to expand the renal lymphatic network in KidVD mice and then induced injury. One week following the AKI, we characterized the renal immune cells by flow cytometry in KidVD mice and their wild type littermates. Our results identified a trend showing a reduction in Th1 type T-cells in KidVD+ mice, while no difference was observed in Th17 or Treg numbers. Renal lymphatics may therefore help to limit the AKI to CKD progression.

This work, GB, and JR were supported by NIH R01 DK119497 to JR. ND was supported by a Fellowship from NIH R25 DK126642.

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CARDIAC MYOSIN BINDING PROTEIN-C PLAYS A ROLE IN REGULATION OF CROSSBRIDGE CYCLING CATALYZING LUSITROPY TO MEDIATE DIASTOLIC FUNCTION

Sarinity Frazier, Abby Leatherman, Lilly McAlister, Carl Tong
Dept. of Medical Physiology
Texas A&M College of Medicine
Bryan, TX

There is no effective treatment for heart failure (HF) with preserved ejection fraction (HFpEF) which accounts for ~50% of all cases of HF. Diastolic dysfunction underlies HFpEF, therefore insight into mechanisms that mediate relaxation can provide new potential targets for novel treatments. We hypothesized that cardiac myosin binding protein C (cMyBPC) plays a significant role in the regulation of crossbridge cycling, thereby catalyzing lusitropy to mediate diastolic function. cMyBPC (-/-) mice were used to identify functional effects of a gene; allowing us to observe the biological system without it present. We compared cMyBPC (KO) *vs* cMyBPC (tWT) in survival, echocardiography, and intact papillary muscle experiments; we used Vevo 3100 system to perform real time echocardiography. Data sets were analyzed using SPSS software. Survival and other analyses found ($P<0.05$) that relative to the tWT, cMyBPC (KO) showed shortened life span. By echocardiography, cMyBPC (KO) hearts exhibited slower e' and higher E/e' to indicate diastolic dysfunction, hypertrophy, reduced ejection fraction, and abbreviated systolic ejection. cMyBPC (KO) mice also exhibited decreased peak calcium to peak force time and abbreviated force duration relative to tWT. This means the KO exhibits accelerated crossbridge cycling and an inability to catalyze relaxation in response to increased pacing frequency due to the lack of cMyBPC.

This work was supported by NIH R01 HL145534 to CT. SF was supported by a Fellowship from NIH R25 DK126642.

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SEX DIFFERENCES IN NOVEL TRANSGENIC MICE WITH CONSITUTIVELY UPREGULATED β2* NEURONAL NICOTINIC ACTEYLCHOLINE RECEPTORS: IMPLICATIONS FOR PARKINSON'S DISEASE

Bruno Hidalgo Monroy Lerma, Gauri Pandey, Sara Zarate, Rahul Srinivasan
Dept. of Neuroscience and Experimental Therapeutics
Texas A&M College of Medicine
Bryan, TX

Chronic tobacco use is correlated with reduced Parkinson's disease (PD) risk, and nicotine is thought to mediate this effect. However, the concentrations of nicotine in chronic tobacco users are too low to activate target neuronal nicotinic acetylcholine receptors (nAChRs), which makes this an unlikely mechanism for neuroprotection of the dopaminergic (DA) neurons lost in PD. We previously showed that nanomolar concentrations of nicotine and the nicotinic ligand, cytisine, chaperone β2-subunit-containing (β2*) nAChRs out of the endoplasmic reticulum (ER), thereby reducing the ER stress response, which results in neuroprotection. To directly test this hypothesis, we created a novel transgenic mouse line called 'β2-mutant', with enhanced ER export of β2* nAChRs. Surprisingly, β2-mutant mice demonstrated significant increases in Sec24D ER exit sites (ERES) within substantia nigra pars compacta (SNc) DA neurons of female, but not male mice. We next compared tyrosine hydroxylase (TH) expression in SNc DA neurons between midbrain sections of β2-mutant and wildtype (WT) littermates. Area of SNc TH labeling served as a measure of somatodendritic morphology, while TH intensity was used as an indirect measure of dopamine synthesis. We found that female, but not male, homozygous β2-mutant mice had significantly higher TH area and expression than WT littermates. These data support sex differences in the relationship between ERES regulation, SNc DA neuron morphology, and TH content in the mouse SNc. Future work will focus on using β2-mutant mice to elucidate mechanisms underlying sex differences in ERES biology of DA neurons, and neuroprotection in a model of PD.

This work was supported by grants from the American Parkinson Disease Association (APDA) and NIH R01 NS115809 to RS. BHML was supported by a Fellowship from the Texas A&M College of Medicine.

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SYSTEMIC IGF-1 TREATMENT EFFECTS ON GUT REPAIR AND ACUTE ISCHEMIC STROKE OUTCOMES

Tiffany Luan, Yumna El-Hakim, Kathiresh Mani, Farida Sohrabji
Dept. of Neuroscience and Experimental Therapeutics
Texas A&M College of Medicine
Bryan, TX

Women experience a rise in stroke risk and severity at postmenopause, a profile linked to estrogen decline. Using acyclic middle-aged female rats that mimic the menopausal stage, we found that estrogen treatment increased infarct volume after stroke, indicating that replacing ovarian hormones was not neuroprotective. Acyclic rats also have lower circulating levels of the neuroprotective peptide hormone Insulin-like Growth Factor (IGF-1), and surprisingly, intracerebroventricular IGF-1 delivery reduced infarct volume and inflammatory cytokine levels in the brain. However, this treatment did not reduce circulating inflammatory cytokine levels or improve long-term depressive behaviors. Evidence that stroke leads to gut dysbiosis and increased gut permeability suggests that targeting the gut via systemic IGF-1 administration, such as an intraperitoneal (i.p.) injection, may be more effective at repairing the gut post-stroke. To test this, acyclic middle-aged (9-11 months) female rats were subject to endothelin (ET)-1 induced middle cerebral artery occlusion (MCAo) or sham surgery and terminated 2d later. I.p. IGF-1 or vehicle injections were administered 4h and 24h post-MCAo. Sensorimotor assessments, blood, and gut samples were obtained pre and 2d post operation. Systemic IGF-1 administration did not reduce infarct volume or attenuate sensorimotor deficit post-stroke. At 2 days, IGF-1 treatment did not show consistent improvement in gut repair; however, peripheral levels of inflammatory cytokines were reduced in preliminary assessments. Since gut repair/regeneration typically has a 3-4 day cycle, these results suggest that future studies should examine stroke outcomes at the late acute stage (5d after stroke) to fully assess IGF-1's reparative properties on the gut.

This work was supported by NIH NS074895, the Discovery Foundation, and Texas A&M College of Medicine. TL was supported by a Fellowship from the Texas A&M College of Medicine.

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HYPERTENSIVE STIMULI INCREASE MURINE LYMPHANGIOGENESIS

Karina Martinez, Brooke Wilcox, Shobana Navaneethabalakrishnan, Bethany Goodlett, Anil Pournouri, Brett Mitchell
Dept. of Medical Physiology
Texas A&M College of Medicine
Bryan, TX

Hypertension affects over 45% of adults in the US and is the largest contributing factor for mortality. Hypertension is also associated with immune system activation and inflammation that can lead to lymphangiogenesis. Lymphangiogenesis is the expansion of the lymphatic vasculature and plays a large role in the resolution of inflammation. Angiotensin II, salt, and asymmetric dimethylarginine induce hypertension, immunity and inflammation, and sodium retention in mice and humans. We hypothesized that these hypertensive stimuli will directly increase lymphangiogenesis. To determine if hypertensive stimuli induce mouse lymphatic endothelial cells (mLECs) to proliferate, mLECs were cultured and treated with hypertensive stimuli for 24 hours and qRT-PCR was performed. Results showed no significant changes in lymphatic gene expression for the lymphatic markers LYVE-1, PROX-1, and podoplanin. To observe the effects of hypertensive stimuli on intact lymphatic vessels, mesometrial vascular beds were excised from PROX-1 tdTomato mice and cultured in hypertensive stimuli with and without conditioned media for 8 days. Conditioned media was prepared by treating splenocytes with hypertensive stimuli for 24 hours. Mesometrial lymphatic vessels treated with hypertensive conditioned media showed a significant increase in average sprout length at day 8 ($p<0.05$). Lymphatic vessels treated with hypertensive conditioned media also had a significant increase in the average number of sprouts at day 8, except for the salt treatment. In conclusion, hypertensive stimuli did not have a direct effect on mLEC proliferation, but the presence of immune cell conditioned media increased lymphangiogenesis.

This work was supported NIH RO1 DK120493 to BM. KM was supported by a Fellowship from NIH R25 DK126642.

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ANALYSIS OF NEK10 AND PALBOCICLIB IN ANGIOGENIC SPROUTING AND CELLULAR PROLIFERATION

Natalia Mejia, Colette Abbey, Ashley Coffell, Kayla Bayless
Dept. of Molecular and Cellular Medicine
Texas A&M College of Medicine
Bryan, TX

Angiogenesis is the process of forming new blood vessels from preexisting vessels, which can be beneficial or detrimental in certain instances. This process involves cellular proliferation, migration, and invasion. Previously in this lab, single cell sequencing was used to analyze the genes expressed in non-invading and invading endothelial cells. Results from multiple data sets showed that Never-In-Mitosis Related Kinase 10 (NEK10) was significantly upregulated in invading cells. In this project, proliferation and invasion were examined using gene knockdown and pharmaceutical drug treatment in invasion assays to better understand the cellular mechanisms of angiogenesis. We hypothesized that the knockdown of NEK10 would impact cellular invasion and proliferation, and Palbociclib would inhibit proliferation in treated cells but would have no effect on invasion. Human endothelial cells were transfected with three different NEK10 siRNAs, and qPCR results showed partial (40%) knockdown of NEK10 for all three. In an invasion assay with siNEK10, there were no significant differences in invasion density in the siNEK10 groups compared to the siβ2M control group. Immunofluorescence staining showed only one siNEK10 sequence group had a slight significant difference with less proliferation than the siβ2M group. When cells were treated with Palbociclib in separate invasion assays, proliferation was significantly inhibited compared to untreated groups. However, there were no significant differences in invasion distance between treatment groups. Further investigation of NEK10 and Palbociclib will be done to gain a better understanding of how each can impact angiogenesis and cellular mechanisms.

This work was supported by a Texas A&M T3 grant to KB. NM was supported by a Fellowship from NIH R25 DK126642.

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IMPACTS OF REACTIVE OXYGEN SPECIES ON CD4$^+$ T CELL SURVIVAL AND METABOLISM

Megan Nitchman, Hao-Yun Peng, Jianxun Song
Dept. of Microbial Pathogenesis and Immunology
Texas A&M College of Medicine
Bryan, TX

CD4$^+$T cells play an essential role in immune protection, including B cell, macrophage, or CD8$^+$T cell activation and releasing cytokines to recruit other leukocytes. Eukaryotic elongation factor 2 kinase (eEF2K) is a translation regulatory protein that negatively regulates peptide elongation. eEF2K is upregulated in some cancer cells and promotes their survival. The role of eEF2K in CD4$^+$T cell activities remain unknown. Studied here were the phenotypic differences between wild-type (WT) and eEF2K knockout (KO) effector CD4$^+$T cells. Preliminary data showed that eEF2K KO CD4$^+$T cells produced significantly more reactive oxygen species (ROS). We hypothesized that increased ROS and decreased expression of antioxidant, superoxide dismutase 1 (SOD1) led to low survival of eEF2K KO CD4$^+$T cells. When treated with ROS inhibitor, N-acetyl cysteine (NAC), the survival and expression of SOD1 in eEF2K KO CD4$^+$T cells should be similar to the WT. Using Western blotting methodology, we report here that the expression of SOD1 in both WT and eEF2K KO populations were statistically similar. The lifespan of WT and eEF2K KO CD4$^+$T cells was measured using trypan blue staining every two days and cells were subcultured on day three. Cells treated with 5mM NAC had better survival than the controls. Additionally, eEF2K KO CD4$^+$T cells have a higher survival, compared to WT, after the treatment of NAC. The culmination implies that T cell survival is decreased due to high generation of ROS and eEF2K lowers ROS, affecting CD4$^+$T cell survival.

This work was supported by Texas A&M College of Medicine and NIH R01 AI121180 and R21 AI128325 to JS. MN was supported by a Fellowship from the Texas A&M College of Medicine.

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T CELL ISOLATION AND ACTIVATION WITH DIFFERENT INDOLE CONCENTRATIONS

Ashley Reytor Plasencia, Jugal Das, Julia Plocica, Darby Ballard, Jianxun Song, Robert Alaniz, Paul de Figueiredo
Dept. of Microbial Pathogenesis and Immunology
Texas A&M College of Medicine
Bryan, TX

Microbiota produce tryptophan metabolites such as indole that have been shown to play a role in regulating the host immune response. Regulating T cells that mediate inflammatory autoimmune disease is a major clinical need. By exposing the harvested cells to different indole concentrations, it is hypothesized that indole will suppress inflammatory T cell responses and increase regulatory T cells (Tregs). In this study, we characterized the effects of indole on T cell activation and effector function with the goal of engineering a bacterial platform to produce indole in vivo as a means to attenuate autoimmune disease. Cells collected from the spleen and lymph nodes of wild-type mice were passed through a 70μL strainer and red blood cell lysis buffer was used to obtain single cells which were then negatively selected through a magnetic column to obtain CD4+CD25- cells. Then antiCD28, antiCD3, and IL-2 proliferate and differentiate the cells in different concentrations of indole and were incubated for three days before intracellular marking was used to determine cell quantity and type. FoxP3 is a transcription factor unique to Tregs and was used in this experiment. Under Treg skewing conditions and increasing indole concentrations, the concentration of Tregs significantly increased compared to the control group. In addition, the pro-inflammatory cytokines IFN-γ decreased and the alternative costimulatory molecule PD-1 decreased compared to the control group under increasing indole concentration. In conclusion, these data provide a strong rationale for engineering indole production into a synthetic microbial platform and may offer a unique and efficacious modality for treating autoimmune diseases.

ARP was supported by a Fellowship from NIH R25 DK126642

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HYPERTENSIVE STIMULI INCREASES LYMPHANGIOGENESIS IN HUMAN LYMPHATIC ENDOTHELIAL CELLS

Anil Pournouri, Brooke Wilcox, Shobana Navaneethabalakrishnan, Bethany Goodlett, Karina Martinez, Brett Mitchell
Dept. of Medical Physiology
Texas A&M College of Medicine
Bryan, TX

Hypertension is the number one contributor to cardiovascular-renal disease and affects almost half of the US population. Hypertension is associated with inflammation, and persistent inflammation results in lymphangiogenesis, the expansion of the lymphatic vasculature. Lymphatic endothelial cells line lymphatic vessels which aid in the trafficking of immune cells and interstitial fluid. We hypothesize that the hypertensive stimuli angiotensin II, salt, and asymmetric dimethylarginine will directly increase lymphangiogenesis. Human lymphatic endothelial cells were cultured and treated with hypertensive stimuli for 24 hours. qPCR revealed there were no changes in gene expression of the lymphatic markers LYVE-1, PROX-1, and podoplanin. To examine lymphangiogenesis, 3-D collagen matrices were made, the treated cells were allowed to invade for 24 hours, and then they were fixed and stained with toluidine blue. When compared to the saline control, hypertensive stimuli significantly increased the number of lumen-forming structures and the invasion distance (both $p<0.05$). However, hypertensive stimuli significantly decreased the average lumen diameter and the number of cells per invading structure (both $p<0.05$). These findings suggest that hypertensive stimuli do not induce cell proliferation; however, they cause an increase in lymphangiogenesis.

This work was supported by NIH RO1 DK120493 to BM. AP was supported by a Fellowship from NIH R25 DK126642.

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DARPIN BINDING TO HA AND HAMINI BY PANNING A PHAGE DISPLAY LIBRARY AGAINST PURIFIED H1N1 PROTEINS

Bianca Rodriguez, Rudo Simeon, Zhilei Chen
Dept. of Microbial Pathogenesis and Immunology
Texas A&M College of Medicine
Bryan, TX

Seasonal influenza poses a significant public health threat, claiming the lives of 3,000 – 49,000 people in the US each year. Since all current FDA-approved treatments of influenza A virus [e.g. oseltamivir (Tamiflu) and zanamivir (Relenza)] need to be administered within 24-48 h post infection in order to be effective, rapid and early diagnosis of influenza infection can both improve the treatment outcome and reduce viral transmission. The overall objective of this project is to engineer a designed ankyrin repeat protein (DARPin) able to bind the H1N1 hemagglutinin (HA) protein for influenza diagnosis. Phage panning was carried using either the full-length HA or the HA stem region (HAmini) as the target protein in alternative rounds. The target protein was biotinylated and phages displaying HA binders are pulled-down by immobilized streptavidin Unfortunately, after 6 rounds of phage panning, little enrichment of DARPin binders to HA or HAmini was observed while the positive control antibody, CR6261, was able to efficiently bind the biotinylated HA and HAmini. In conclusion, we failed to enrich HA-specific DARPins. One possible reason for the failure is the partial denaturation of the target protein during prolonged storage. In the future, freshly purified proteins or unmodified proteins will be used in the phage panning.

BR was supported by a Fellowship from NIH R25 DK126642.

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PREACTIVATION OF MAST CELL LIMITS THEIR ACUTE RESPONSE

Diksha Shakya, Morgan Jackson, Anatoliy Gashev
Dept. of Medical Physiology
Texas A&M College of Medicine
Bryan, TX

A high density of mast cells (MCs) is found in all tissues directly interacting with the external environment. These immune cells are sensitive to various microenvironmental changes. These changes induce certain levels of MCs activation, further leading to their degranulation with subsequent release of numerous bioactive mediators. These mediators modulate the surrounding tissues and initiate immediate immune/inflammatory responses. In addition, MCs might be pre-activated by the low-grade inflammation. Such may diminish the MCs responses when foreign challenges affect the body. In this study, we assessed the ability of the pre-activated MCs to respond to an acute challenge. This study included 4 cohorts for both male and female SD rats; that received intraperitoneal injections of 48/80 compound, cromolyn sodium, and lipopolysaccharide (LPS) in different combinations. The 48/80 acts as a chemical MC activator, the cromolyn sodium acts as a MC stabilizer, and the LPS mimics infection-related MC activation. Influences of various treatment regimens on blood pressure and heart rate were examined using an MRBP machine specially designed for rats. Intensity of the MCs' activation and degranulation were evaluated microscopically in mesenteric tissue segments, stained with Alexa Fluor 488-conjugated avidin, followed by toluidine blue staining. Samples of serum and lung tissue were collected for determinations of MCs-derived mediators. Preliminary data demonstrate expected trends, but more analyses and experiments are under way.

This work was supported by DOD DM190495 to AG. DS was supported by a Fellowship from NIH R25 DK 126642.

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EXPLORING THE ROLE OF CMPK2 IN MTDNA MAINTENANCE AND MITOCHONDRIAL METABOLISM

Mary Stahlman, Sylvia Torres-Odio, A. Phillip West
Dept. of Microbial Pathogenesis and Immunology
Texas A&M College of Medicine
Bryan, TX

Mitochondrial dysfunction can play a key role in disease, aging, and infections. Release of mitochondrial constituents such as mitochondrial DNA (mtDNA) and ATP into the cytosol stimulates the innate immune system and promotes pathology in several diseases. CMPK2 is a complex mitochondrial protein described to be involved in mtDNA maintenance and induced after immune challenges. Though some studies have described CMPK2's role in viral infections, the specific function of CMPK2 in mitochondrial homeostasis at steady state and after challenge remains unknown. The objective of this project is to explore the role of CMPK2 in mtDNA maintenance and mitochondrial metabolism at rest and after lipopolysaccharide (LPS) challenge. CMPK2 protein is present in the lungs and brain but not in liver and muscle of WT mice at baseline. Analysis of mtDNA copy number and Oxidative Phosphorylation (OXPHOS)-related proteins showed no significant difference in WT and CMPK2-KO mice in liver and lungs before and after LPS challenge. *In vitro* analysis of immortalized bone marrow derived-macrophages indicated higher levels of OXPHOS proteins at baseline and soon after LPS challenge in CMPK2-KO cells. Similarly, CMPK2-KO cells had higher maximal respiratory capacity and spare respiratory capacity than WT cells with no changes in glycolysis status. These results indicate that CMPK2 does not play a role in mtDNA maintenance but seems to be involved in mitochondrial metabolism. Future directions include metabolic assessment of primary cells and tissues of WT and CMPK2-KO mice to advance our understanding of CMPK2 function in mitochondrial homeostasis.

MS was supported by a Fellowship from the Texas A&M College of Medicine.

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CHRONIC ALCOHOL INTAKE IMPAIRS BEHAVIORAL FLEXIBILITY IN THE REVERSAL OF INSTRUMENTAL LEARNING

Madison Wang, Matthew Childs, Yufei Huang, Xueyi Xie, Zhenbo Huang, Jun Wang
Dept. of Neuroscience and Experimental Therapeutics
Texas A&M College of Medicine
Bryan, TX

Alcohol use disorder (AUD) is a chronic brain disorder characterized by an inability to control alcohol use despite adverse consequences. Evidence suggests that thalamic inputs to dorsomedial striatum (DMS) cholinergic interneurons (CINs) play an important role in behavioral flexibility. Our previous studies showed that chronic alcohol intake reduced thalamic excitatory inputs to DMS-CINs and impaired behavioral flexibility in reverse instrumental learning. In this project, we aimed to rescue this behavioral inflexibility by optogenetic manipulation which could enhance thalamic inputs to DMS-CINs. Two-bottle choice alcohol drinking and alcohol vapor chamber sessions established a history of chronic alcohol intake prior to behavior training. The rats were trained in operant chambers to learn left lever presses associated with a sucrose solution reward and right lever press associated with a food pellet reward. Then, rats were trained with reversed contingencies (left lever press with food reward and right lever press with sucrose) to assess behavioral flexibility. Before reversal training, half of the rats received *in vivo* optogenetic stimulation via optic fibers implanted in the DMS. The rats demonstrated goal-directed behavior following initial training but were unable to do so after reversed contingencies training, suggesting optogenetic stimulation was unsuccessful at rescuing impairment of behavioral flexibility. This reduced behavioral flexibility may facilitate compulsive alcohol use in patients with AUD. Understanding how chronic alcohol intake affects CINs in the striatum and behavioral flexibility will allow for the development of therapeutic approaches to treat AUD in the future.

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TEXAS A&M HEALTH
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2021 SUMMER UNDERGRADUATE RESEARCH PROGRAM

PHYSIOLOGICAL AND BEHAVIORAL INDICATORS OF FEAR LEARNING

Colin Wei, Jony Sheynin, Samira Ahrari, Israel Liberzon
Dept. of Psychiatry
Texas A&M College of Medicine
Bryan, TX

Post-traumatic stress disorder (PTSD) is a mental health condition, supplemented with feelings of danger, and heightened arousal, even in contexts where no threat is present. In order to study contextual processing, healthy individuals (*n=25*) underwent a fear-learning paradigm. Fear conditioning (light paired with loud noise) occurred in one context, followed by extinction (same color light paired with no noise, E) in another, to create danger and safety contexts, accordingly. Each context had two interleaving colors of lights, one associated with the noise (CS+) and another that isn't (CS-). On the following day, the extinguished conditioned stimulus (CS+E) was presented again to assess extinction recall (ER) and fear renewal (FR) in safe and dangerous contexts, respectively. Participants' physiological responses were assessed using skin conductance response (SCR), and their behavioral responses were assessed by recording their reported expectancy of a loud noise. We found no difference in skin conductance between CS+E and CS- during early ER, but higher CS+E in late ER. During FR, the participants displayed a greater SCR to CS+E. Behaviorally, subjects expected a loud noise from CS+E relative to CS- in ER, regardless of context. FR displayed no differences. Physiologically, participants appropriately recalled safe and dangerous contexts in early ER and FR. Behaviorally, participants showed impaired learning of ER and FR. In the future, the study will recruit PTSD patients and compare their performance with the healthy individuals reported here to identify the effect of PTSD on contextual processing.

CW was supported by a Fellowship from the Texas A&M College of Medicine.

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COXIELLA BURNETII MODULATES HOST CELL NF-KB PATHWAY DURING INFECTION

Ricardo Alfaro Zeledon, Saugata Mahapatra, James Samuel
Dept. of Microbial Pathogenesis and Immunology
Texas A&M College of Medicine
Bryan, TX

Coxiella burnetii is a gram negative obligate intracellular bacterium that causes the zoonotic disease Q Fever. Human disease typically occurs via bacterial inhalation, where bacteria infect host monocyte/macrophages. Experiments in our lab show that C. burnetii utilizes a functional Type IVB Secretion System (T4SS) to modulate the host innate immune transcription factor Nuclear Factor-κB (NF-κB) during infection. Stimulation of C. burnetii infected THP-1 Lucia cells with PAM3CSK4, a TLR2 agonist, shows that bacteria block NF-κB activation in a T4SS-dependent manner. In this study, we used THP-1 cells to examine whether C. burnetii employs its T4SS to inhibit the gene expression of NF-κB-driven pro-inflammatory cytokines IL6 and TNFα during infection. Non-adherent THP-1 cells were grown in RPMI media and infected with either C. burnetii NM II or NMIIΔdotA, a T4SS-defective mutant, at an MOI of 25. Cells were stimulated with 0.1, 1 and 2.5 ng/ml of PAM3CSK4 at 2 hours post infection (hpi) and total RNA was harvested at 6hpi and 12hpi using Trizol. After RNA extraction, samples were DNAsed to remove any residual genomic DNA present. Next, cDNA was synthesized from each sample and (+/-) reverse transcriptase PCRs performed with primers for IL-6 to account for DNA contamination. Currently, RT-qPCRs are being performed to analyze the differential expression of IL-6 and TNFα in THP-1 cells infected with either C. burnetii NM II or NMIIΔdotA. We anticipate that results from these experiments will corroborate our preliminary observations suggesting that C. burnetii utilizes its T4SS to inhibit TLR-induced NF-κB activation during infection.

This work was supported by NIH to JS. RAZ was supported by a Fellowship from the Texas A&M College of Medicine.

2021 Texas A&M College of Medicine
Summer Research Program Participants

Joseline Alvarez	Texas A&M University – San Antonio	Dr. David Zawieja
Darius Caldwell	Prairie View A&M University	Dr. Sarah Bondos
Naomi Dillard	Prairie View A&M University	Dr. Joseph Rutkowski
Sarinity Frazier	West Texas A&M University	Dr. Carl Tong
Bruno Hidalgo Monroy Lerma	Georgia Tech University	Dr. Rahul Srinivasan
Tiffany Luan	Baylor University	Dr. Farida Sohrabji
Karina Martinez	West Texas A&M University	Dr. Brett Mitchell
Natalia Mejia	Texas A&M University – Kingsville	Dr. Kayla Bayless
Megan Nitchman	Lycoming College	Dr. Jim Song
Ashley Reytor Plasencia	Tarleton State University	Dr. Robert Alaniz
Anil Pournouri	Texas A&M University – Texarkana	Dr. Brett Mitchell
Bianca Rodriguez	Texas A&M University – Kingsville	Dr. Zhilei Chen
Diksha Shakya	Texas A&M University – Commerce	Dr. Anatoliy Gashev
Mary Stahlman	University of Alabama	Dr. Phillip West
Madison Wang	University of Texas – Austin	Dr. Jun Wang
Colin Wei	Rice University	Dr. Israel Liberzon
Ricardo Alfaro Zeledon	Texas A&M University	Dr. James Samuel

2021 Texas A&M College of Medicine
Summer Research Program Seminar Series

Date	Time	Topic	Presenter
5/24	9:00 AM	Orientation, Kaplan, and Laboratory Safety Training	
5/27	9:00 AM	URM Roundtable Discussion	Dr. Dianne Kraft
5/28		Memorial Day Holiday	
6/1	12:00 PM	CST*R Grand Rounds	MD/PhD Students
6/3	9:00 AM	URM Roundtable Discussion	Dr. Dianne Kraft
6/4	9:00 AM	RCR – Data Management	Dr. Brett Mitchell
6/8	12:00 PM	RCR – Animal Subjects	Dr. Farida Sohrabji
6/10	9:00 AM	URM Roundtable Discussion	Dr. Dianne Kraft
6/11	9:00 AM	RCR – Transparency & Reproducibility	Dr. Brett Mitchell
6/15	12:00 PM	TAMU College of Medicine MD Program	Mr. Filomeno Maldonado
6/17	9:00 AM	URM Roundtable Discussion	Dr. Dianne Kraft
6/18	9:00 AM	Roundtable – Writing an Abstract	Dr. Brett Mitchell
6/22	12:00 PM	TAMU College of Medicine MS/PhD Program	Dr. Carol Vargas
6/24	9:00 AM	URM Roundtable Discussion	Dr. Dianne Kraft
6/25	9:00 AM	Roundtable – Marketing and Interviewing	Dr. Brett Mitchell
6/29	12:00 PM	TAMU College of Medicine MD/PhD Program	Dr. Carolyn Cannon
7/1	9:00 AM	URM Roundtable Discussion	Dr. Patricia Watson
7/2	9:00 AM	Roundtable – Organizing Your Poster	Dr. Brett Mitchell
7/6	12:00 PM	A&M Rural and Community Health Institute	Dr. Nancy Dickey
7/8	9:00 AM	URM Roundtable Discussion	Dr. Dianne Kraft
7/9	9:00 AM	Roundtable – Giving a 10 Minute Talk	Dr. Brett Mitchell
7/13	12:00 PM	RCR – Scientific Misconduct	Dr. Vernon Tesh
7/15	9:00 AM	URM Roundtable Discussion	Dr. Dianne Kraft
7/16	9:00 AM	Roundtable – Presenting at Conferences	Dr. Brett Mitchell
7/20	12:00 PM	Southwest Rural Health Research Center	Dr. Alva Ferdinand
7/22	9:00 AM	URM Roundtable Discussion	Dr. Dianne Kraft
7/23	9:00 AM	Roundtable – Applying to School	Dr. Brett Mitchell
7/26	9:00 AM	Student Presentations	
7/27	9:00 AM	Student Presentations	
7/28	9:00 AM	Student Presentations	
7/30	9:00 AM- 2:00 PM	Research Day (Poster Presentations, Reception, Keynote Speaker, Awards Ceremony, and Pictures)	

Program Director

Dr. Brett Mitchell, Ph.D., FAHA

Department of Medical Physiology
Texas A&M Health
College of Medicine
MREB II, Rm. 2412
Bryan, TX 77807
Email: brettmitchell@tamu.edu
Phone: 979.436.0751

PLEASE KEEP US UPDATED WITH YOUR CONTACT INFORMATION AND CAREER OR SCHOOL DECISIONS AFTER GRADUATION.

THANK YOU AND THANKS FOR YOUR HARD WORK THIS SUMMER!

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