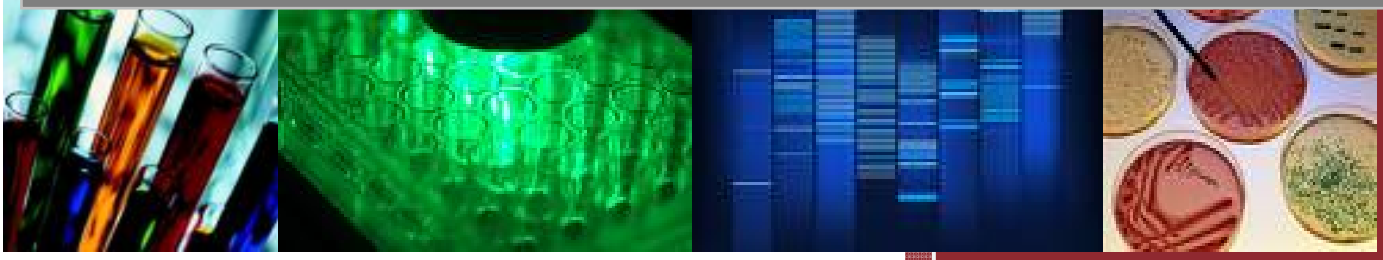
Poster Session and Reception

2011

Summer Research Program



August 17, 2011

9:00am-2:00pm

Health Professions Education Building

Bryan, TX

TEXAS A&M
HEALTH SCIENCE CENTER
COLLEGE OF MEDICINE

Program

August 17, 2011

8:30am	**Registration Table Opens** HPEB L1 Lobby
9:00am-2:00pm	**Poster Presentations** HPEB LL43 A&B
11:30am	**Lunch** HPEB L1 Lobby
12:15-1:00pm	**Keynote Speaker** Dr. Clifford C. Dacso, "The New World of Translational Community Medicine" HPEB LL46
1:00-1:30pm	**Presentation of Certificates** Dr. Warren Zimmer, Director of the Summer Research Program HPEB LL46
1:30-1:45pm	**Presentation of Dean's Recognition Awards** HPEB LL46
2:00pm	**Adjourn**

Speaker's Biography

Clifford C. Dacso, MD, MPH, MBA
Founder and Executive Director of the Abramson Center for the Future of Health Institutes of Health Translational Medicine
Baylor College of Medicine and Methodist Research Institute
Houston, Texas 77030

Clifford C. Dacso, MD, MPH, MBA,

has been helping patients and clinicians acquire, structure, and use information to improve healthcare for over 35 years. He is the founder and Executive Director of the Abramson Center for the Future of Health: a research center that leverages technology, clinical medical expertise, and theoretical mathematical decision theory to support individuals as the primary managers of their own health. In the Center's first six years, he recruited a cadre of young aggressive investigators who shared his mission of integrating social sciences, technology, and community-based care, in the United States and internationally.

Dr. Dacso's research and development focuses on new technologies that integrate information and support for patients with chronic disease, particularly in under-resourced communities. He holds two patents for wireless, non-invasive devices for management of chronic diseases such as congestive heart failure, asthma, and diabetes. The prototype of his non-invasive sensor for measuring heart rate and muscle oxygenation during exercise recently entered clinical trials, and the Center's serious game for decision making in localized prostate cancer is at the beta stage of development. Dr. Dacso's work with developing countries includes co-founding CRASA (Centre de Recherche et Amélioration de la Santé en Afrique) and research on Buruli ulcer and breast cancer detection and prevention.

This work has resulted in three patent applications, two National Science Foundation grants, two NIH grants, a Texas Emerging Technology Fund grant, a 2010 Goradia Innovation Award, a Microsoft

Imagine Cup prize and numerous publications and presentations at national and international conferences. The Center has also formed collaborations with researchers in England, Ireland, Mexico, and Israel.

Dr. Dacso is the John S. Dunn Sr. Research Chair in General Internal Medicine, Professor of Molecular and Cellular Biology at Baylor College of Medicine, Professor of Clinical Medicine at Weill Cornell Medical College, Hugh Roy and Lillie Cranz Cullen University Professor at University of Houston, Adjunct Professor of Electrical and Computer Engineering at Rice University, and a Consultant to the Biological Diagnostic Institute of Dublin City University. He holds degrees from the University of Pennsylvania (BA, MA 1972), Baylor College of Medicine (MD 1975), University of Texas (MPH, 1980) and Pepperdine University (MBA 1990). He was a 1999 Eisenhower Fellow.

Acknowledgements

For the second year we limited the number of participants in the SRP to thirty students (20 medical and 10 undergraduate), which has resulted in the SRP being the "hottest ticket in town" – not an editorial on the local weather! Our application numbers were again strong (~4 applications/spot) and the quality of applications equally strong, making the competition for a spot in the program especially keen (please see a list of the participants on page 39). The SRP committee evaluated each application and with an NIH study section-like meeting came to a consensus on which students to offer a spot. This task is becoming more difficult each year and I applaud the committee for their diligent work. An interesting by product of limiting places in the program has been individual investigators and groups such as Texas Brain and Spine asking if their sponsored students could participate in the talks/program – even presenting posters at today's reception. I think this speaks to the high quality of the faculty participants in the program, both as mentors and presenters of stimulating/informative lectures (please see page 40 for a listing of the speakers and topics). They are the real VIPs of the program and deserve a hardy "job well done". The program would not be able to sustain its quality and existence without them.

Even though we serve two distinct student populations, medical students between their first and second years and undergraduates from around the country, all students participate equally in the program. This enriches the experience as each group can learn from the other and share their ups and downs of laboratory experience. Each has worked extremely hard this summer and the posters displayed at today's reception are the products of their hard work. Please take the time to visit the posters and ask the students what they did during their summer vacation; be prepared to be amazed by their work and abilities!

We obtained funding from a number of sources and would like to thank Dr. Shomaker, College Dean; Dr. Wesson, Vice Dean of the Temple Campus (Scott and White Research); and Dr. David Carlson, Vice President for Research and Graduate Studies for major contributions to our budget. In addition, we could not have had a successful year without the generous support of Dr. Roy Smythe, Chairman of Surgery; Dr. Alejandro Arroliga, Chairman of Medicine, and Dr. Harris Granger, Chairman of Systems Biology and Translational Medicine. It is difficult, but not impossible, to provide simultaneously to three locations. The work of Drs. Murray (College Station), Mitchell (Temple) and Huston (Houston) as site coordinators keeping things running efficiently is greatly appreciated. Finally, I would like to thank Dr. Van Wilson and his staff in College Station, **Rachel Levins, Mary Ann Wolff, Jordan Sulsar, Stephen O'Shea, and Emily Sterling;** Dr. Huston's staff in Houston, **Anna Wirt and Karol Franks;** and the Dean's staff in Temple**, Loria Lynce and Cari Cummings** for making certain that the entire program got off the ground and running effortlessly.

Warren Zimmer, PhD
Director, Summer Research Program

Abstracts

TAMHSC SUMMER RESEARCH PROGRAM

August 17, 2011 9:00 AM - 2:00 PM
Health Professions & Education Building
Bryan, TX

Pre-Manipulative Anesthetic in Lumbar Microdiscectomy

Gerard Toussaint III, Ramesh Kumar, Nathan Bertoldo, Austin Adair
Department of Neuroscience and Experimental Therapeutics
Texas A & M University Health Science Center College of Medicine, (College Station)

Introduction

In a lumbar microdiscectomy procedure, the nerve root must be pushed aside to gain access to the herniated disc portion for excision. The normal procedure is to apply nothing to the nerve root before it is manipulated. However, several recent studies indicate that possibly steroids or anesthetic applied to the nerve root may decrease post-operative pain levels.

Hypothesis

We predict that administration of bupivacaine directly on the nerve root before it is pushed aside will help decrease post-operative pain levels and decrease post-operative pain medication use.

Methods

Patients will be enrolled in the study at our office. We will have a form for study participants sent over with the pre-operative admission papers so that the hospital contact may receive a copy. We can also have a second person in the room, such as the circulator or scrub tech, when the card is drawn. The scrub tech and circulator will know what groups are indicated by the letters. They will then draw up the appropriate solution and hand it to the surgeon at the appropriate time.

We will measure the pre-operative pain in the day stay area using the VAS pain survey and then again at discharge, 2 weeks, and 2 months post-operatively. The differences between the pre-operative pain rating and each subsequent interval pain rating will be recorded. All patients should be given the same pain medication at the same PRN regimen post-operatively. The amount of narcotics given will be recorded by the nurse. Length of stay will be measured by recording arrival time discharge time from PACU. We will attempt to enroll 150 patients for safe measure.

Results

Due to a low patient enrollment thus far, it is undetermined whether the added step in the microdiscectomy procedure proves useful. The study will be continued in the future to increase the number of enrollees.

TAMHSC SUMMER RESEARCH PROGRAM

August 17, 2011: 9:00 AM - 2:00 PM
Health Professions Education Building
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Substance P and TNF- α mediated modulation of inflammatory cytokine expression and the PI3K/AKT pathway in the lymphatics

Abdulhamid Al-Douri, Sanjukta Chakraborty, Mariappan Muthuchamy
Systems Biology and Translational Medicine
Texas A & M University Health Science Center College of Medicine, College Station, TX

Introduction: The lymphatic system is the one of the most vital systems in our body. It aids the immune system by destroying pathogens and filtering waste, as well as maintaining fluid balance, protein homeostasis, and immune function. The involvement of lymphatic pumping function in edema resolution, immune cell trafficking, and their sensitivity to inflammatory mediators make them pivotal players in the process of inflammation. Any defect to the lymphatic system can result in a wide range of disorders. The system's task of propelling lymph is vital for maintenance of fluid balance. The system is also responsible for transporting antigens to lymphoid tissues to allow initiation of an immune response during disease and infection. It is well established that the lymphatic system is intimately involved and highly altered during inflammation. An increase in lymph flow is thought to occur during inflammation due to an increase in vascular permeability and the resultant interstitial fluid. Lymph flow can also be disrupted by a change in the lymphatic muscle pumping, which results from inflammation. Substance P (SP) and TNF- α are potent mediators of an inflammatory response and previous studies have implicated them as modulators of lymphatic function. However, the specific inflammatory pathways and the resultant cytokines activated by SP and TNF- α is not known in the lymphatics. In this study, we attempt to better understand the inflammatory response activated by these two agents in the lymphatic muscle cells.

Hypothesis: We hypothesized that SP and TNF- α would stimulate the pro-survival PI3K/AKT pathway, known to be involved in cellular proliferation and inflammatory responses, and would increase the production of pro-inflammatory cytokines by activation of inflammatory mediators, such as NF-κB and I-κB in the lymphatic muscle cells.

Methods: Cultured rat mesenteric lymphatic muscle cells (RMLMC) was used as a model system. RMLMC were plated in complete DMEM media and grown at 37°C in a 10% CO_2 incubator. The cells were treated with SP (1μM) or TNF- α (20ng/ml) at different time points. After the drug treatment, the cells were lysed at the specific time points; both RNA and protein were isolated. The proteins were separated by SDS-PAGE, and the gels were transferred to nitrocellulose membranes. Western analysis was carried out to detect the expression of phospho-AKT, total AKT, phospho-I-κB, total I-κB, pFOXO1, and total FOXO1. RNA isolated from the treated cells was quantified and the cytokine expression (IL-1β, IL-4, IL-6, IL-10, c-fos and MIP-2) was evaluated using RT-PCR analysis.

Results: Our results indicate that both SP and TNF-α differentially regulate the expression of the key players of the PI3K/AKT pathway and activate expression of phospho I-κB and NF-κB in a time dependent manner in the RMLMC. We also show a modulation of inflammatory cytokine expression patterns when the cells are stimulated by SP or TNF-α. Taken together our data suggest that differential regulation of the PI3K/AKT pathway activates the expression of several pro-inflammatory cytokines and further studies using specific pharmacological inhibitors is in progress to clearly delineate the role of key molecular players of this pathway in mediating an inflammatory response in the lymphatics.

TAMHSC SUMMER RESEARCH PROGRAM

August 17, 2011 9:00 AM - 2:00 PM
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The role of ssRNA in the development of preeclampsia

Allen S, Chatterjee P, Doersch K, Chiasson V, Narayanan A, Young K, Kopriva S, Mitchell B
Department of Internal Medicine
Texas A & M University Health Science Center College of Medicine, Temple Campus

Introduction: Preeclampsia (PE) affects approximately 10% of all pregnancies and is characterized by hypertension and proteinuria at or after 20 weeks gestation. We have shown previously that dsRNA induces PE-like symptoms in mice through the activation of the innate immune system receptor Toll-like receptor (TLR) 3. However, the role of ssRNA (derived from viruses and/or necrotic cells) and its receptors TLR7/8 in the development of PE is unknown.

Hypothesis: We propose that the ssRNA mimetics R837 and CLO97 trigger a maternal immune response that elicits PE-like symptoms in mice.

Methods: Mice were divided into 6 groups: 1) C=non-pregnant controls treated with vehicle, 2) CR=non-pregnant treated with R837 (TLR7 agonist), 3) CC=non-pregnant treated with CLO97 (TLR 7/8 agonist), 4) P=pregnant controls treated with vehicle, 5) PR= pregnant treated with R837, and 6) PC=pregnant treated with CLO97. Treatments were given by ip injection on days 13, 15, and 17 of gestation or corresponding days in non-pregnant mice, and tail cuff blood pressures were measured on days 13 and 17. Mice were sacrificed on day 18 of gestation and aortas, sera, urine, spleens, placentas, and pups were harvested. Endothelium-intact aortic segments were tested for endothelium-dependent and –independent dilation and urinary protein excretion was analyzed by the pyrogallol red total protein assay.

Results: R837 and CLO97 significantly increased spleen/body weight values in non-pregnant mice (C: 4.5 ± 0.54 mg/g, CR: 5.3 ± 1.6 mg/g, CC: 4.9± 1.0; CR and CC $p<0.05$ vs. C) as well as pregnant mice (P: 3.5 ± 0.4 mg/g, PR: 4.3 ± 0.4 mg/g, PC: 4.5 ± 0.6 mg/g; PR and PC $p<0.05$ vs. P). However, for all non-pregnant mice there were no significant changes in blood pressure, vascular reactivity, or urinary protein excretion. In pregnant mice, R837 and CLO97 significantly increased systolic blood pressure (P: 104 ± 1.4 mmHg, PR: 128 ± 5 mmHg, PC: 131 ± 4 mmHg; PR and PC $p<0.05$ vs. P). Endothelium-dependent aortic relaxation responses were significantly decreased in PR and PC mice compared to P. Endothelium-independent aortic relaxation responses were comparable in all 6 groups. Urinary protein excretion was increased significantly in PR mice compared to P; however, there was no change in PC mice. R837 and CLO97 had no effect on total number of offspring; however, fetal demise was significantly increased in PR and PC mice.

Conclusions: Based on these findings, it is apparent that ssRNA mimetics trigger an immune response. However, only pregnant mice developed hypertension and endothelial dysfunction. Although excessive TLR7 and TLR8 activation during pregnancy increased fetal demise, only TLR7-specific activation caused proteinuria during pregnancy. From a clinical perspective, these findings indicate the potential for TLR 7/8 antagonists as treatments for hypertensive disorders of pregnancy.

TAMHSC SUMMER RESEARCH PROGRAM

August 17, 2011 9:00 AM - 2:00 PM
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Characterization of protein-protein interactions between retinoic acid-induced 2 and Nkx3.2

Douglas K. Armour and Warren E. Zimmer
Department of Systems Biology and Translational Medicine
Texas A & M University Health Science Center College of Medicine, (College Station)

Introduction

We investigated the protein-protein interactions of a poorly understood protein retinoic acid-induced 2 (Rai2) and homeobox domain containing protein, Nkx3.2. Both retinoic acid responsive molecules (like Rai2) and homeobox proteins are vital in normal development and can play a role in diverse pathologic conditions. Rai2 was discovered in 1999 by a Walpole *et.* al studying Nance-Horan syndrome (cataract-dental deformity developmental syndrome) and was found to be highly homologous to mouse retinoic acid-induced gene product. Rai2 is highly conserved in sequence and in its location on the X-chromosome; this conservation of structure and genetics indicates that it is vital to development and disease. No one has set out to understand the precise pathways that Rai2 modulates and interacts with. Our lab has worked extensively with NK factors; Nkx3.2, a potent regulator of mesenchymal development, has been studied. Sonic hedgehog is a vital developmental controller and a powerful inducer of Nkx3.2.

Methods

We utilized a yeast two-hybrid screen to find proteins that potentially interact with Nkx3.2. Among the strongest of the binding proteins identified in our library screen was Rai2. In addition, we have used pair wise analyses in yeast to demonstrate a direct interaction between these two molecules. Thus, we have shown that these two proteins do interact, but we want to evaluate the interaction in a mammalian system that more closely mimics the native cellular environment. To accomplish this we devised a co-transfection system where expression vectors are placed into cultured COS 7 cells and the expression of the Rai2 and Nkx3.2 proteins are verified by western analyses. We then used an HA-tag added to the Rai2 molecule to immunoprecipitate this protein, and we analyzed the precipated products for the presence of Nkx3.2. To map which regions of Nkx3.2 bind Rai2, we utilize mutants of Nkx3.2 and evaluate lysates from cells which have been transiently transfected with Nkx3.2 (wild type and mutants) and Rai2 by the immunoprecipitation assay. This work is vital to laying the groundwork to understanding the interactions of Rai2 with other proteins and begins to establish a foundation for understanding how these two systems could cross-talk; moreover, this strong interaction may act as site of integration and convergence of these two distinct developmental pathways.

TAMHSC SUMMER RESEARCH PROGRAM

August 17, 2011 9:00 AM - 2:00 PM
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Pharmacodynamics of Human Recombinant Arginase I [Co]-PEG5000

Gabriel Axelrud, Keri Kleypas, Shu-Ru Kuo, Jen-Sing Liu, Arthur Frankel
Cancer Research Institute, Scott & White Hospital, Temple, Texas
Texas A&M Health Science Center College of Medicine, Temple, Texas

Introduction

Cancer cells dependent on the extracellular availability of amino acids for growth have been observed in a number of different malignancies. Depletion of such needed amino acids should inhibit auxotrophic tumor growth and, therefore, represents a selective anti-cancer treatment. Arginine is a semi-essential amino acid synthesized from arginosuccinate by arginosuccinate lyase (ASL). Arginine auxotrophic neoplasms are deficient in cytoplasmic arginosuccinate synthetase (ASS), the enzyme responsible for the rate limiting conversion of citrulline and aspartate to arginosuccinate in the urea cycle. It has been shown that arginine deprivation is effective against solid tumors that fail to express the ASS enzyme. L-citrulline co-administration may further improve the therapeutic index since this amino acid only requires the presence of ASS and arginosuccinate lyase to replenish cellular arginine stores, thereby protecting normal tissue that is unable to rescue arginine from the amino acid ornithine. Human recombinant arginase I cobalt (HuArgI[Co]) coupled to polyethylene glycol 5000 (HuArgI[Co]-PEG5000) has shown potent *in vitro* depletion of arginine from tissue culture media. HuArgI[Co]-PEG5000 in combination with supplemental L-citrulline is selectively cytotoxic to a fraction of human cancer cell lines in tissue culture including some melanomas, mesotheliomas, acute myeloid leukemias, hepatocellular carcinomas, pancreas adenocarcinomas, prostate adenocarcinomas, lung adenocarcinomas, osteosarcomas, and small cell lung carcinomas. It has been observed that i*n vivo* treatment of BALB/c mice with HuArgI[Co]-PEG5000 results in bone marrow depletion with absence of damage to other major organs; however, when L-citrulline is co-administered, the combination partially alleviates the toxicities associated with high doses of HuArgI[Co]-PEG5000.

Hypothesis

HuArgI[Co]-PEG5000 in combination with L-citrulline supplementation may be an attractive therapeutic agent for ASS deficient tumors. The goal of this project is to analyze the serum of HuArgI[Co]-PEG5000-treated BALB/c mice using high performance liquid chromatography (HPLC) that involves precolumn derivatization with *o*-phthaldialdehyde (OPA) in order to quantify post-treatment arginine levels.

Methods

Freshly obtained plasma is immediately deproteinized and neutralized. The HPLC analytical column (SUPELCOSIL™ LC-18) is washed with water and methanol (15 minutes each) and then equilibrated with mobile phase A (0.1 *M* sodium acetate, pH 7.2) for at least 20 minutes at a flow rate of 1.1 mL/min before running a sample set. Mobile phase B consists of 100% methanol in a brown bottle. Analysis is started by mixing 50 μL of the sample solution with 50 μL of OPA reagent solution (30 m*M* OPA, 50 m*M* 2-mercaptoethanol, 40 m*M* sodium borate, and 3.1% Brij-35, pH 9.5) for 1 minute and delivering the derivatized solution into the HPLC column without any delay time. Analysis run time is 49 minutes at a flow rate of 1.1 mL/min in a gradient elution program. Fluorescence is monitored at excitation and emission wavelengths of 340 and 455 nm, respectively.

Results

The results show that HuArgI[Co]-PEG5000 effectively lowers arginine levels of *in vitro* treated rat and mouse serum as quantified by HPLC. *In vivo* results are pending completion and serum acquisition from BALB/c mice groups treated with HuArgI[Co]-PEG5000.

TAMHSC SUMMER RESEARCH PROGRAM

August 17, 2011 9:00 AM - 2:00 PM
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Chemotactic Effects of CCL2, CCL3, CXCL-1, and GM-CSF on Hippocampal Neural Stem Cells and Embryonic Primary Hippocampal Cells

Jade Booher[1,2,3], Cody Ruhl[1,2],Clarissa Cavarsan[1,2], Sanjib Mukherjee[1,2], Lee Shapiro[1,2,3]
1, Scott & White Hospital; 2, VA Central Texas Health Care System; 3,
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Introduction

More than one million people suffer traumatic brain injuries (TBIs) every year in the United States (Dempsey et al. 2009) and there are as many as 50,000 annual deaths contributed to TBIs. In addition, the sequelae associated with a TBI can be quite devastating. Not only may a TBI patient suffer long term cognitive deficits, such as memory loss and attention problems, but there are also behavioral and affective issues associated with TBIs: including irritability, anxiety and depression. TBIs have also been found to increase seizure susceptibility and give rise to post-traumatic epilepsy (PTE), which is defined as recurrent, spontaneous seizures that occur more than a week following a TBI. It is estimated that PTE accounts for 20% of symptomatic epilepsy in the general population (Agrawal et al. 2005) but despite this prevalence, there are currently no effective interventions due to the incomplete understanding of the pathogenesis of PTE. Recently, it has been shown that the pathophysiology of TBIs induce an inflammatory and immunological response (Schmidt et al. 2004). Inflammation is induced by various types of tissue injuries and consists of the rapid production of molecules with pro-inflammatory or anti-inflammatory properties by cells of the immune system, including both innate and adaptive immunity cell types (Vezzani, 2011). In fact, if this inflammatory process is very severe or prolonged, the inflammatory reaction itself can actually cause tissue damage or dysfunction. It is this immunological process that is thought to be part of the underlying pathophysiology of PTE. Neural plasticity and reorganization have been found to develop during a latent phase after a TBI and more specifically, aberrant growth and migration of newborn neurons occurs. It is thought that integration of these cells may contribute to the increased seizure susceptibility that ensues after a TBI and it is possible that these neuroanatomical changes may be linked to certain inflammatory cytokines that are up-regulated after a TBI. Because of these potential consequences, elucidating the possible pathogenic effects of these inflammatory molecules is an emerging area of interest. Levels of CCL2 (also known as monocyte chemoattractant protein or MCP-1), CCL3 (also known as macrophage inflammatory protein alpha or MIP-1α), CXCL-1 (also known as GRO/KC), and granulocyte-macrophage colony stimulating factor (GM-CSF) were found to be increased 24 hours after a lateral fluid percussion injury (FPI) was inflicted in rats (Mukherjee et al. 2011). CCL2 recruits monocytes, memory T cells, and dendritic cells to sites of tissue injury, infection, and inflammation. CCL3 is best known for its chemotactic and pro-inflammatory effects but can also promote homoeostasis. CCL3 is also known to be increased in temporal lobe epilepsy, head injuries, and other neuropathologies. CXCL-1 has neutrophil chemoattractant activity, plays a role in spinal cord development by inhibiting the migration of oligodendrocyte precursors, and is also involved in the processes of angiogenesis, inflammation, wound healing, and tumorigenesis. GM-CSF appears to be neurotrophic, neurogenic, and neuroprotective after a central nervous system injury. In the current study, we are looking at the chemotactic effects of these inflammatory cytokines on both neural stem cells and primary hippocampal cells in vitro.

Hypothesis

To test if the cytokines CCL2, CCL3, CXCL-1, and GM-CSF exert chemotactic effects on neural stem cells and/or primary hippocampal cells in vitro.

Methods

Cell Culture:

Constitutive GFP Reporter Adult Rat Hippocampal Neural Stem Cells (Millipore) – cells were cultured according to the protocol provided by the supplier

E18 Primary Rat Hippocampal Cells (Neuromics) – cells were cultured according to the protocol provided by the supplier.

Migration Assay: QCM Chemotaxis Cell Migration Assay (Chemicon) was performed on each cell type with different cytokines (CCL2, CCL3, CXCL-1, and GM-CSF) at four different concentrations. The Contitutive GFP Reporter Adult Rat Hippocampal Neural Stem Cells were incubated with the cytokines and were then analyzed for migration after 3 hours, 6 hours, and 24 hours. The E18 Primary Rat Hippocampal Cells were incubated with the cytokines and were then analyzed for migration after 6 hours. Medium without any cytokines was used as a control for each time point.

Quantification: The QCM Chemotaxis Cell Migration Assay protocol was followed and fluorescence was read using a fluorescence plate reader with a 480/520 nm filter set.

Results

Results of the migration assay will be discussed in the context of inflammation-induced aberrant neural plasticity.

TAMHSC SUMMER RESEARCH PROGRAM

August 17, 2011 9:00 AM - 2:00 PM
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Bryan, Texas

Determining ligands for *Clostridium difficile* surface proteins

Tyler Buchanan, Magnus Höök, Caná Ross
Center of Extracellular Matrix Biology, Institute of Biosciences and Technology,
Texas A&M University Health Science Center College of Medicine, Houston, Texas

Introduction: Surface-anchored proteins from Gram-positive bacteria interact with a variety of extracellular matrix proteins, including fibrinogen, fibronectin, vitronectin, collagen, and laminin. It is likely that these interactions mediate colonization of the host. *Clostridium difficile* is a Gram-positive, spore-forming bacterium that is the major cause of antibiotic associated diarrhea (AAD), and infection with *C. difficile* can lead to pseudomembranous colitis, toxic megacolon and death. Infection with *C. difficile* usually occurs after treatment with antibiotics, which disturb the normal gut flora. Current research efforts into *C. difficile* pathogenesis focus largely on the toxins which are responsible for the intestinal damage. However, less is known about how *C. difficile* mediates attachment to the intestinal epithelia.

Hypothesis: Putative surface-anchored *C. difficile* proteins bind to extracellular matrix and basement membrane proteins.

Methods: Expression: TOPP3 *Escherichia coli* cells containing the IPTG-inducible expression vector harboring DNA encoding a portion of a putative *C. difficile* surface protein were inoculated into Laura Bertani broth containing carbenicillin (100μg/ml). Following overnight growth at 30° C, cells were subcultured at a dilution of 1:100 into fresh LB containing carbenicillin (50μg/ml) and incubated at 37° C with shaking. Exponentially growing cells were induced with IPTG (200 μM). Three hours post-induction, cells were harvested by centrifugation (20 min, 4500rpm). Cell pellets were resuspended in phosphate buffered saline (PBS) containing protease inhibitor and lysed using a French Press at 1100 p.s.i. Cellular debris was removed by ultra-centrifugation at 40,000 rpm for 20 minutes at 4° C. The supernatant was filtered through a 0.45 μM membrane, and purified using a Nickel-charged chelating column. Bound proteins were eluted with imidazole. Fractions containing the protein of interest were dialyzed into Tris HCl pH=7.4 and applied to a MonoQ anion exchange column, and were eluted with NaCl. Fractions containing the His-tagged recombinant protein were concentrated and dialyzed into PBS containing 0.5M EDTA, pH=7.4.

ELISA-type assay: Immulon 4HBX plates were coated with ligands resuspended in PBS overnight at 4° C. The plates were blocked with 2% BSA in Tris-buffered saline containing 0.05% Tween (TBS-T) for 1 hour at room temperature (RT). Wells were probed with increasing concentrations of purified protein for a 1-hour at RT. Bound proteins were detected with His-HRP antibody (1:3000). *SigmaFast* OPD and a microtiter plate reader were used to detect binding.

Results and Conclusion: *C. difficile* protein 1-4-20, the putative ligand-binding domain of a *C. difficile* surface protein, exhibits concentration-dependent binding to type I collagen (TIC). These data demonstrate that this protein segment specifically interacts with TIC. It is possible that this protein mediates attachment of *C. difficile* to human intestine via interactions with TIC.

TAMHSC SUMMER RESEARCH PROGRAM

August 17, 2011 9:00 AM - 2:00 PM
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Harnessing the Cytotoxicity of Human Eosinophils against Cancer Cells

Soumili Chatterjee, Zhaleh J. Amini-Vaughan, Kathleen T. Nguyen,
Ghamartaj Tavana and David P. Huston
Clinical Science and Translational Research Institute
Texas A & M University Health Science Center College of Medicine, (Campus- Houston)

Introduction

Eosinophils are a subpopulation of granulocytes that only account for 0-5% of circulating leukocytes, but have profound and varied roles in immune and inflammatory responses. Physiologically, eosinophils respond to helminth infections by secreting granules containing cytotoxic effector molecules such as major basic protein (MBP). Eosinophils are also a biomarker of allergic inflammation and are pathogenic for hypereosinophilia. Therefore, antagonizing eosinophils are a viable therapeutic strategy for these disorders. **Given the cytotoxic potential of eosinophils, this study investigates the hypothesis that human eosinophils can be harnessed against tumor cells.**

Aims

To test the hypothesis that human eosinophils can be used as an immunotherapeutic agent against tumor cells *in vitro*.

Methods

ATCC human prostate cancer cells were maintained in culture. Human eosinophils were isolated from leukopacks via Robosep negative antibody selection. Eosinophil purity was determined by dual staining for CD16 and CD66 by flow cytometry. Eosinophils were co-cultured with the prostate tumor cells the following day in varying effector-target cell ratios. The co-culture was incubated for 4 hours and viability was assessed by cell count and flow cytometry using Annexin V and 7-AAD staining.

Results

From the flow cytometry generated graphs and data, we saw that in the co-culture the tumor cell (PC3 cell) count decreased in a dose dependent manner. The tumor cell count in the 1.25:1 co-culture was 6054, whereas it became 5914, 4873 and 3471 in 5:1, 10:1 and 20:1 co-cultures respectively. The viability was consistent across the doses as evidenced by the Annexin V and 7-AAD staining.

Discussions

The data indicates that prostate cancer cell count is reduced when co-cultured with eosinophils in a dose dependent manner. This indicates that the eosinophils are targeting the tumor cells and inducing apoptosis. Therefore, it can be concluded that human eosinophil cytotoxic properties can be harnessed against tumor cells in an immunotherapeutic manner and be applied *in vivo*. Future studies will investigate the effects of eosinophils against other cancer cell lines, such as lung and colon cancer cells, as well as determine the specificity and mechanism of human eosinophils cytoxicity. We will also explore the potential to harness eosinophill effector function *in vivo* using a mouse model.

TAMHSC SUMMER RESEARCH PROGRAM

August 17, 2011: 9:00 AM - 2:00 PM
Health Professions Education Building
Bryan, TX

Towards Evaluation of the Interaction between CDK8 and SREBP during Lipogenesis

Jae H. Cho, Hao-Ching Hsiao, Sarah E. Bondos, Jun-yuan Ji
Department of Molecular and Cellular Medicine
Texas A & M University Health Science Center College of Medicine, College Station

Introduction

Most proteins have distinct structures due to their unique amino acid sequence, enabling each protein to have a single discrete function. However, intrinsically disordered proteins lack stable structure, and their structure and function can be altered by cellular factors. This flexibility allows the intrinsically disordered proteins to have multiple context-specific functions. One of the possible mechanisms for context-specific function is post-translational modifications, such as phosphorylation. To study phosphorylation as a mechanism to regulate context-specific function, we studied sterol regulatory element-binding protein (SREBP), a protein whose activity is regulated by phosphorylation *in vivo*. SREBP is an intrinsically disordered protein which functions as a transcription factor that regulates sterol synthesis. It is synthesized as a precursor protein that is inserted into the ER membrane. The membrane bound form of SREBP is inactive, and it is activated when it is processed by site-directed proteolysis. The processed mature form of SREBP is translocated into the nucleus and functions as a transcription factor. SREBP binds to its target site as a dimer, and the dimer formation is believed to be disrupted by a phosphorylation that alters the structure of SREBP.

Hypothesis

Previous studies suggest that CDK8 is involved in regulation of SREBP activity. CDK8 knock-out resulted in 2-8 fold up-regulation of genes that are regulated by SREBP. We hypothesize that CDK8 regulates SREBP activity either by inhibiting dimer formation or by recruiting cellular machinery that leads to degradation of SREBP. Both models require direct interaction of CDK8 with SREBP, and this study aims to provide molecular evidence for this protein-protein interaction.

Methods

To test for an interaction between CDK8 and SREBP, both proteins must be recombinantly synthesized. To do so, DNA encoding the *Drosophila* nuclear form of SREBP (dnSREBP) was inserted into *Escherichia coli* to generate a large quantity of dnSREBP protein. Different media and time periods were tested to optimize the production of dnSREBP, which was monitored by Western Blot using anti-his-tag antibody to detect the his-tag present in the vector (pET-19b). dnSREBP protein was purified by Ni-NTA chromatography. dnSREBP and dCDK8 co-expression was also tested to see if direct binding of two proteins is observed. Due to difficulties in expressing dCDK8, the dCDK8 cDNA was sub-cloned into a different vector (pGEX6-p2). Site-directed mutagenesis was performed on the vector to generate NdeI and NotI restriction sites needed for sub-cloning.

Results

Expression of dnSREBP in 2xYT and LB Luria with 0.1mM $FeSO_4$-$7H_2O$ and 0.4% glucose showed greater expression of dnSREBP than in LB Luria and LB Miller, but excessive amount of protein degradation products was observed. Expression of dnSREBP in LB Luria for 3 hours yielded a high amount of dnSREBP (0.684 mg/mL) with small amount of degradation products. dnSREBP protein ran at higher than expected length on a SDS-PAGE gel, potentially due to dimer formation of dnSREBP. Indeed, the same result is also observed in previous study. Mass spectrometry will be used to confirm that the protein is dnSREBP. The protein is successfully purified, so when the dCDK8 protein is produced after sub-cloning into pGEX6-p2, direct interaction of dnSREBP and dCDK8 will be tested by mixing the purified proteins to observe direct binding.

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Lysophosphatidic Acid (LPA) Stimulates MG-63 Osteosarcoma Cells to Invade Three-Dimensional (3D) Collagen Matrices in a MT-MMP Dependent Manner

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Introduction

Cellular invasion of the extracellular matrix is essential for many physiological and pathological processes, such as angiogenesis and tumor growth or metastasis. A number of events are necessary for invasion, including response to a stimulus, activation of integrins, activation of proteinases such as matrix metalloproteinases (MMPs), cytoskeletal mobilization. Sphingosine -1phosphate (S1P) and lysophosphatidic acid (LPA) are extracellular lipid agonists that induce tumor cell proliferation, migration, and invasion. Previous work has linked soluble MMPs such as MMP-1,-2,-3,-9,-10, and -13 to tumor invasion and metastasis. Recent studies, however, suggest that membrane-type MMPs (MT-MMPs) are necessary for the focused ECM proteolysis that occurs during invasion. Osteosarcoma (OSA) is common primary bone tumor in children, adults, and domestic animals. Although OSA originates from bone, OSA cells may metastasize, leading to additional distant pathology. To our knowledge, the role of S1P, LPA, and the MMPs in invasion of osteosarcoma cells in three-dimensional (3D) type I collagen has yet to be evaluated.

Hypothesis

We hypothesized that LPA and S1P would stimulate MG-63 cells to invade 3D collagen matrices and that membrane-type MMPs (MT-MMPs) would be required for invasion.

Methods

MG-63 cells were cultured in DMEM, 10% fetal bovine serum, and maintained at 37ºC with 5% CO_2. Prior to each experiment, cells were washed with PBS, trypsinized, neutralized with FBS, and centrifuged. Cells were washed in an additional 10 ml volume of DMEM to remove residual serum. 3D collagen gels (n= 3 gels/condition, 25 µl volume) were prepared at a final concentration of 2.5 or 3.75 mg/ml in A/2 wells of 96 well plates. D-Erythro-sphingosine-1-phosphate (S1P) or lysophosphatidic acid (LPA) were incorporated into gels prior to polymerization and equilibration at 37ºC and 5% CO2 for 45 minutes. After equilibration, 100 µl of media containing a 1:250 dilution of RSII, 50 µg/ml ascorbic acid, and 25,000 MG-63 cells were added to each well. In some instances, proteinase inhibitors were added to the culture media as follows: GM6001 (5 µM), TAPI-0 (5 µM), aprotinin (20 KIU/ml), and TIMP-1 or TIMP-3 (5 µg/ml). Upon termination of experiments, cells and gels were fixed in 3% glutaraldehyde in PBS prior to staining with 0.1% toluidine blue in 30% methanol for photography and quantification of invasion. Individual invading cells were counted with an eyepiece equipped with an ocular grid. For each condition, 20 fields were selected within each well and invading cells were counted at 20X. Data are reported as mean number of invading cells per HPF (+/- S.D.). Conditioned media were collected and analyzed via gelatin zymography or Western blot analysis, and cell lysates were prepared for Western blots.

Results

LPA markedly stimulated invasion of MG-63 cells in a dose-dependent manner, whereas S1P had minimal effects. Increasing the concentration of type I collagen from 2.5 to 3.75 mg/ml reduced MG-63 invasion, suggesting that the density or composition of the surrounding matrix played an active role in this process. The chemical MMP inhibitors GM6001 and TAPI-O dramatically inhibited invasion, whereas addition of the serine proteinase inhibitor aprotinin had no effect. Addition of TIMP-1, an inhibitor of soluble MMPs, had no effect on invasion, while addition of TIMP-3, an inhibitor of both soluble and membrane-type-MMPs (MT-MMPs) dramatically inhibited invasion, suggesting that MT-MMPs are required for invasion of 3D collagen matrices.

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DETERMINING THE POTENTIAL OF DOMAIN V TO INFLUENCE NEURITE EXTENSION IN VITRO

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Introduction

Tissue plasminogen activator (t-PA) remains the sole FDA licensed ischemic stroke therapy. Because of the limited treatment options for stroke, Dr. Gregory Bix's lab has focused its research on identifying potential factors that could be both neuroprotective and enhance the brain's own post-stroke reparative mechanisms. The lab recently determined that the c-terminal fragment of the extracellular matrix component perlecan, termed domain V (DV), is neuroprotective, promotes angiogenesis, and acutely improves motor function when exogenously administered 24 hrs after focal cerebral ischemia. However, the complete mechanisms underlying DV's potential as a stable long-term stroke treatment remain undetermined.

New neurons mobilized after ischemic injury will attempt to migrate to the damaged area, extend neurites, and make synapses thereby becoming functional neuronal replacements. Neurite extension/neuritogenesis is a critical process in the differentiation and establishment of new neurons into their environment. These processes ultimately become the neuronal axon and dendrites.

Therefore, investigating treatments that may enhance the neurite outgrowth process are highly significant for long-term stroke recovery.

Hypothesis

We hypothesize that DV treatment will result in the enhancement of neurite extension and synaptic connection formation in vitro.

Methods

Fetal cortices were dissected from E15-17 mice in cold MHBSS in a 60mm petri dish. The MHBSS was aspirated, the cortices were cut with a surgical blade, and 5 mL trypsin was added to the dish. The contents were transferred to a 50 mL conical tube and incubated at 37°C for 5 minutes. 5 mL 10% FBS GM was added to the conical, and the cells were triturated. After being poured into a new 50 mL conical tube through a 100 μm cell strainer, the cells were placed in a centrifuge for 5 minutes at 1000 rcf. The media was aspirated, and the cell pellet was resuspended in 1 mL of B27/DMEM. The cells were counted at a 1:20 dilution and then plated onto Poly-D-Lysine coated 24 well plates. The plates were incubated overnight at 37°C, 5% CO_2. Half of the wells were treated with 300 nM DV in BASAL/DMEM media, while the other half was only treated with BASAL/DMEM media. At 2, 4, 8, and 24 hours, neurons were fixed with 4% paraformaldehyde and stained with 1% Cresyl Violet. The plates were viewed by simple microscopy.

Results

Simple microscopy revealed both increased neurite extension and synaptic connection in the DV treated wells at 2, 4, and 8 hours.

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The role of perlecan and domain V in the angiogenic and invasive biology of glioblastoma

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Introduction

Glioblastoma multiforme (GBM) remains a uniformly fatal cancer, despite advances in surgical and pharmaceutical interventions. The ability of GBM to co-opt normal brain vasculature, generate its own profound vascularity, and invade along vessels in Virchow-Robin spaces are major contributing factors to the lethality of this disease. The role of the vascular extracellular matrix in fostering invasiveness via the perivascular tumor stem-cell niche is unknown. Preliminary data has suggested enhanced perivascular expression of perlecan (an extracellular matrix proteoglycan) in xenograft models of GBM. Furthermore, domain V (DV, the C-terminal fragment of perlecan that is activated by proteolysis from full length perlecan) appears to increase motility in non-malignant glial cells. DV has also been indicated in the enhancement of brain angiogenesis in a VEGF dependent manner. The primary goal of this study is to demonstrate the importance of perlecan and DV in the invasiveness and overall biology of GBM.

Hypothesis

Through this study, we expect to demonstrate that perlecan, and its cleaved DV, have unique functional roles in the maintenance of critical GBM phenotypes - invasion and angiogenesis.

Methods

In order to illustrate enhanced expression of perlecan and/or DV in the perivascular space of GBM samples, immunohistochemical staining and fluorescent imaging of both mouse xenograft and human clinical GBM samples were performed. Half of the tissue sections received DV and vWF antibodies, while the others received perlecan and DV antibodies. Any non-tumorous brain tissue in the xenografts were used as controls. To define the correlation of perlecan and DV expression to invasiveness, eight GBM cell lines were grown in culture. These include U87, U373, U251, which are human GBM cell lines, and C6, a rat glioma cell line. From each of these four lines, subpopulations with differing invasive phenotypes than the parent lines were generated (U87-IM3, U373-IM3, U251-IM3, and C6-IM3). In the coming weeks, when each of these cell cultures has reached adequate confluence, both lysates and conditioned media from all cell lines will be collected. Western blots will then be performed, probing the membranes for DV. ImageJ gel analysis will be used to quantify the western blots, enabling comparison of DV expression between the cell lines of varying invasiveness. B-actin will be used as the loading control. Finally, in order to determine the functional significance of perlecan and DV presence on tumor capillary morphogenesis (in primary tumor cultures), tissue from the operative resection of GBM will be added to T-75 flasks, each pre-coated with different extracellular matrix components, including purified perlecan, DV, laminin, fibronectin, and collagen IV. Capillary-like growth will be quantified in 10hfps per flask and compared between substrates. Because this aim requires resected GBM tissue to be immediately transported to the lab for culture in the coated flasks, its completion is dependent on a local patient undergoing an operative GBM resection, which has not yet occurred during the length of this project.

Results

The human clinical GBM sections have shown high levels of perlecan and DV expression in perivascular spaces, which is especially apparent in the areas of dense, abnormal tumor vasculature. Further staining of the GBM xenografts is currently being done in order to better compare the levels of perivascular DV expression in tumorous tissue to that of the surrounding nontumorous tissue. The western blots for DV will offer a more quantitative insight into the relationship between DV expression and GBM invasiveness. However, due to the unexpectedly slow growth of the GBM cells in culture, results from the western blots are not expected for one to two more weeks.

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Anti-apoptotic Effect of Retinoid Receptor Signaling in Diabetes-induced Cardiac Remodeling

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Introduction: *Diabetes mellitus* is a key risk factor for cardiovascular diseases. Cardiomyocyte apoptosis is considered to be one of the major factors involved in diabetic cardiomyopathy. Retinoic acid (RA), the active metabolite of vitamin A, has been shown to have cardio-protective effects in response to various stimuli. Activation of the retinoic acid receptor (RAR) and retinoid X receptor (RXR) also has an anti-diabetic effect; but, the role in cardiac remodeling remains unclear. Recently, our findings have shown that down-regulation of RARα and RXRα promotes high glucose-mediated cardiomyocyte apoptosis. Activation of RAR/RXR signaling protects cardiomyocytes by reducing oxidative stress and this effect contributes to the anti-apoptotic effect of retinoids. Nuclear factor (NF)-κB has been well characterized in the context of the heart; but, its role in diabetic cardiomyopathy is not yet elucidated. We explored mechanisms by which retinoid signaling can be cardio-protective by regulating NF-κB signaling in diabetic cardiomyopathy. In the present study, by using neonatal rat ventricular myocytes (NRVMs) and an animal model for Type 2 Diabetes (Zucker Diabetic Fatty rat), we determined that the anti-apoptotic effects of retinoic acid are mediated by regulating NF-κB signaling.

Objective: To elucidate the mechanism of the anti-apoptotic effect of retinoid receptor signaling in diabetes-induced cardiac remodeling.

Methods: For *in vitro* studies, NRVMs were treated with high glucose (HG; 25 mM). Nuclear translocation of NF-κB was analyzed by Western blotting. Cardiomyocytes were pre-treated with JSH-23, a pharmacological NF-κB inhibitor for 1 h, and then treated with HG for 24 h, and the change in pro- and anti-apoptotic proteins detected by Western blot analysis. *In vivo* studies were performed in Zucker Diabetic Fatty rats (ZDF) and Lean Zucker rats. The rats were treated with RXR agonist (LGD1069; 20 mg/kg body weight) and RAR-α agonist (Am580; 1 mg/kg body weight) to activate retinoid signaling. Western blot analysis of heart tissue lysate was performed to determine the retinoic acid mediated regulation of NF-κB activation and apoptosis in diabetes.

Results & Conclusions: Activation of RAR and RXR inhibits apoptotic signaling in the diabetic heart. High glucose stimulation increased nuclear translocation of NF-κB, which was attenuated by RA, suggesting that the protective effects of RA may be regulated through NF-κB signaling. A decreased expression of anti-apoptotic proteins (Bcl-X_L and Bcl-2) and increased expression of pro-apoptotic protein (Bax) were prevented by JSH-23 in a dose-dependent manner, indicating that NF-κB mediated signaling has an important role in high glucose-induced apoptosis. Activation of retinoid receptor signaling in the diabetic rat inhibits cardiac IκBα phosphorylation, and thus may suppress NF-κB activation mediated apoptosis. In summary, our findings suggest that retinoid receptor signaling inhibits apoptosis by blunting NF-κB activation in the diabetic heart.

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Excessive placental Toll-like receptor 7/8 signaling contributes to human and experimental preeclampsia

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Introduction

Preeclampsia (PE) is a pregnancy-related disorder that complicates about 10% of pregnancies and leads to health risks for both mother and fetus. While the exact causes of PE remain elusive, recent studies suggest involvement of the immune system, specifically the innate components residing at the maternal-fetal interface that respond to danger signals. It is unknown whether the single stranded RNA receptors TLR7 and TLR8 in the placenta are involved in the development of PE.

Hypothesis

We hypothesized that placentas from women with PE have increased TLR7 and TLR8 activation and NFkB (a mediator of inflammation) expression and that stimulation of TLR7/8 would cause inflammation in human cytotrophoblast (CTB) cells and PE in mice.

Methods

TLR7/8 expression was measured in human placentas from PE and normotensive women by immunohistochemistry (IHC). CTBs were treated with R837 (which stimulates TLR7) and CL097 (which stimulates both TLR7 and TLR8) for 6, 24 or 48 hours and TLR7 and TLR8 activation was analyzed by qPCR and Western blot. Placentas from mice treated with R837 or CL097, which exhibit PE-like symptoms, were analyzed by Western blot and qPCR.

Results

Women with PE had a significant increase in placental TLR7, TLR8 and NFkB expression compared to normotensive women. R837- and CL097-treated CTBs showed increased TLR7 at 6 and 24 hours. Mice treated with CL097 and R837 showed a significant increase in placental TLR7 activation by qPCR (3-fold) and TLR7, TLR8 and NFkB levels by Western blot. Placentas from mice treated with CL097 showed a significant 2.5-fold increase in TLR8 by qPCR.

Conclusions

These results suggest that excessive TLR7/TLR8 activation plays a role in PE and inhibition of this signaling pathway may reduce the severity of PE.

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Disease-Modifying Effect of the Synthetic Neurosteroid Ganaxolone in the Hippocampus Kindling Model of Epileptogenesis

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Introduction: Epilepsy is a chronic condition characterized by recurrent unprovoked seizures. Currently, there is no specific drug for preventing or curing epilepsy. Neurosteroids are steroids produced within the brain via sequential reductions of steroid precursors. Due to positive allosteric interaction with the GABA-A receptors, neurosteroids enhance the tonic and phasic inhibition in the brain and thereby reduce epileptogenesis: the process whereby a normal brain becomes progressively epileptic because of precipitating factors and seizures seen in limbic epilepsy. Ganaxolone is a synthetic analog of the neurosteroid allopregnanolone that has superior features including improved bioavailability and safety profile. The main goal of this study is to determine the efficacy of neurosteroids, including synthetic analogs, in suppressing epileptogenesis.

Hypothesis: The specific aim of this research is to determine the efficacy of the synthetic neurosteroid ganaxolone on the development of limbic epilepsy in the hippocampal kindling model of epileptogenesis. It is hypothesized that ganaxolone, which augments GABAergic tonic inhibition, inhibits or retards the progression of epileptogenesis.

Methods: Experiments were conducted in adult mice using a hippocampal kindling model. A bipolar electrode was surgically implanted into the hippocampus of mice using stereotactic coordinates from the bregma. After a recovery period of 10 days, after-discharge threshold (ADT) was determined for each mouse. Animals were stimulated daily at 125% of ADT and behavioral and electrographic seizures were recorded after stimulation. The mice were divided into two test groups (n=8). One group received a daily dosage of 1 mg/kg, 15-min prior to stimulation while the other group received a daily dosage of 3 mg/kg, 15-min prior to stimulation. Epileptogenesis was evaluated by monitoring the progression of behavioral seizures (Racine scale, stage 0 to 5) and AD duration. AD duration was recorded using the Axoscope software. Ganaxolone was also tested in fully-kindled mice to assess its ability to suppress behavioral seizures.

Results: Ganaxolone produced robust antiseizure effects in fully-kindled mice. Initial results indicate that, at low (1 mg/kg) and medium (3 mg/kg) dose, ganaxolone caused marked retardation of development of behavioral and electrographic seizures in mice. Studies are under progress to quantify the kindling retarding effect of ganaxolone.

Conclusions: The results of this preliminary experiment show that ganaxolone has promise in delaying or retarding epileptogenesis in a mouse kindling model. Further studies are needed to confirm these pilot observations. ** Supported by NIH grant NS051398 **

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The Structural Basis for Binding of BSP-Binding Protein to Human Fibrinogen

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Introduction

Staphylococcus aureus is an opportunistic pathogen capable of causing a number of illnesses, ranging from mild skin infections to life-threatening sepsis. Virulence of the bacteria can be traced in part by its ability to bind to a variety of host extracellular matrix proteins. MSCRAMMS, Microbial Surface Components Recognizing Adhesive Matrix Molecules, are a class of proteins that aid in attachment and other virulence mechanisms. A subfamily of MSCRAMMs can be found in the Sdr family. Structurally similar,all contain a signal sequence, a ligand-binding A region divided into three subdomains (N1, N2, and N3), a B-repeat region, a serine-aspartate dipeptide repeat (Sdr) region, and a membrane-spanning region. BSP-Binding Protein (Bbp) is a member of the Sdr family that has recently been found to bind to the human clotting protein, fibrinogen (Fg). Bbp binds to Fg via the "Dock, Lock, & Latch" mechanism, in which the N2 and N3 subdomains form a ligand-binding trench for Fg to dock. The N3 subdomain has a flexible extension that covers the trench and inserts itself into the β-sheet of the N2 subdomain.

In order to confirm the structure as elucidated recently by the Bbp-N2N3 and Fg co-crystal, point mutations were made in the critical binding region of Bbp using site-directed mutagenesis, then were expressed and purified using affinity purification. The ability of the Bbp mutants to bind to human Fg were assessed using ELISA-type assays. Further characterization of the interaction was accomplished using peptide inhibition ELISA-type assays.

Hypothesis

Creating point mutations in the critical binding region of Bbp, the locking region at the C-terminus of the N3 subdomain, will decrease its ability to bind to fibrinogen.

Methods

1. *Site-Directed Mutagenesis* was used to create point mutations in the locking region found at the C-terminus of the N3 subdomain of the Bbp peptide. Three different mutants were made based on recommendations by crystallographer Ganesh Vannakambadi: Leucine 584 Valine, Serine 585 Threonine, Threonine 586 Serine.

Primers containing the appropriate point mutations were used in site-directed mutagenesis to create and amplify mutated Bbp plasmids. The mutant Bbp plasmid sequences were confirmed and transformed via heat shock into TOPP3 *E. coli* cells. These strains were grown up for large-scale expression of protein and induced overnight with IPTG.

2. *Affinity Purification*

1 liter cultures used for expression were centrifuged with the subsequent cell pellets being lysed with a French Press. The cellular lysate was ultracentrifuged to pellet the cell membranes and other cellular debris. The supernatant, containing the protein of interest, was run over a HisTrap column and eluted with imidazole in order to attain 90-95% purity according to SDS-PAGE. The fractions were collected, dialyzed into a salt-free buffer, and run over a Q-column in order to obtain an approximately 98% pure protein sample.

3. *ELISA-Type Assay and Peptide Inhibition ELISA-Type Assay*

In the ELISA-type assays, plastic wells were coated with human and animal Fg, and varying concentrations of Bbp were added to the wells. Detection was accomplished through a Bbp-specific primary antibody, HRP-conjugated secondary antibody and the SigmaFAST OPD kit. Bbp binding to Fg was measured at 450 nm with a UV/Vis spectrophotometer. In the peptide inhibition ELISA-type assay, Bbp was incubated with a 15mer peptide from wild-type human Fg that is responsible for binding, then added to wells coated with human Fg. Lack of Bbp binding to the wells indicated that the Bbp was already bound to the wild-type human Fg.

Results

Initial Bbp screens using ELISA-type assays were done with human and animal Fg, showing that Bbp binds specifically to human Fg. Peptide inhibition ELISA-type assays confirmed that the wild type human Fg peptide inhibited Bbp binding to human Fg. Mutated Fg peptides showed varying levels of inhibition according to peptide inhibition ELISA-type assays. Data on ELISA-type assays using the mutated Bbp proteins is still being collected.

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Characterization of Biomarkers in Diastolic Dysfunction

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Introduction

Diastolic dysfunction encompasses abnormalities in left ventricular (LV) relaxation, distensibility or filling. These abnormalities can exist regardless of the ejection fraction or symptoms. Diastolic dysfunction leading to diastolic heart failure is equally prevalent as patients with systolic failure and carries the same morbidity and mortality in this subset of patients. However mechanisms underlying diastolic dysfunction are still poorly understood. Currently, diastolic dysfunction is identified by echocardiography and characterized as 4 stages of increasing severity. Tissue Doppler parameters are used to stage severity of diastolic dysfunction. This study addresses the utility of a new biomarkers in identifying different stages of diastolic dysfunction.

Aim:

To assess the utility of biomarkers in diagnosis of diastolic dysfunction

Hypothesis

We hypothesize that the new tissue Doppler parameter Ea/Sa will be useful in distinguishing the grades of diastolic dysfunction using 2D echocardiography

Research Methods

Two hundred eighty one echocardiograms consisting of the four stages of diastolic dysfunction were analyzed and standard tissue Doppler parameters including Ea/Sa were analyzed statistically. Correlations were carried out between tissue Doppler parameters and clinical markers such as blood glucose, hemoglobin A1c, lipid profile and BNP.
Statistical Analysis: Primary analysis of variance to determine variation of Ea/Sa in the four stages of diastolic dysfunction was performed. As a secondary analysis, we determined if Ea/Sa varies with clinical markers such as blood glucose, hemoglobin A1c, lipid profile and BNP.

Results

The results suggest a statistically significant correlation between echocardiographic parameters and stages of diastolic dysfunction hence serving as markers of progression of diastolic dysfunction.

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Membrane Vesicles as a New Vaccine Platform for Gram-Negative Bacterial Infections

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Introduction: Vaccines continue to be a crucial method of preventative medicine, and therefore it remains imperative to further investigate pathogen components and their biological/immunogenic properties for intrinsic vaccine potential. One such upcoming vaccine method employs the use of outer membrane-vesicles (MVs). MVs are dynamic nano-particles, composed of lipids and proteins derived from the bacterial periplasm and outer membrane of gram-negative bacteria. They are involved in a various number of bacterial processes including secretion, biofilm formation, and normal membrane degradation. Due to their dense composition of bacterial generated proteins, lipids, and polysaccharides, these particles mirror bacterial cells in their antigen makeup and can therefore potently stimulate both innate and adaptive immunity. However, these particles do not contain functional DNA or replication machinery so they cannot replicate and cause infection. This research focused on the stimulatory capacity of MVs obtained from wild type, WaaZ null, and sdiA mutants of *Salmonella typhimurium* on mouse derived dendritic cells (DCs). DCs were chosen because this cell type is known to be the most stimulatory of the antigen presenting cells of the innate immune system and thus serves as a gateway cell to eliciting strong humoral and adaptive immune response. Additionally the two mutants were chosen because they both have mutations in genes that alter the structure and frequency of bacterial components that contribute to the outer membrane antigenic makeup. Modifications to the unique extracellular architecture of antigens can profoundly change a cell's or particle's pro-inflammatory effects on immune system cells. WaaZ is a gene that is involved in LPS biosynthesis with deletion of this gene leading to significant truncation of the most exterior domain of this lipid, the O-antigen. LPS is known to be one of the most immunogenic antigens on bacterial cell surfaces and thus alterations in its structure are thought to diminish its stimulatory ability. sdiA is thought to be involved in regulating the production of fimbrae, a structure that allows bacteria to attach to host cells and other bacteria thereby mediating virulence.

Hypothesis: MVs pro-inflammatory properties are genetically manipulable via modification of specific outer membrane components. Specifically, after exposing DCs to MVs harvested from wild type *Salmonella typhimurium*, a marked increase in TNF-α (a known inflammatory mediator produced by stimulated DCs) production is expected on flow cytometric analysis. However, when comparing the stimulations levels of the two mutants being analyzed we expect modified TNF-α production when compared to that of wild type-MV treated DCs. The WaaZ mutant is predicted to cause decreased stimulation due to its alterations of the crucial LPS antigen. The sdiA mutant stimulatory profile is a little more unpredictable due to some of its undefined functions. However, an up regulation of fimbrae would potentially lead to an increase in stimulatory ability due to the increase in the antigenic subunits that makeup this structure.

Methods: All MV preparations began as 35ml of TSB broth and the desired bacterial inoculums. After 24hrs of incubation these starter cultures were added to 1 L of TSB broth and allowed to incubate for 4 hours while shaking. The culture was then centrifuged to obtain a cell-free supernatant which was then filtered using 0.2 nanometer pore-size filters. This filtered supernatant was further purified using 100,000 kDa Millipore exclusion filters to remove smaller particles such as flagella monomers leaving a concentrated MV retentate. The retentate was collected and then ultra-centrifuged at very high speeds to pellet out the MVs. The MV pellet was resuspended in sterile water, frozen, and then later lyophilized to achieve a measurable dry weight. The MVs were finally reconstituted to 1mg/ml in sterile water and stored at 4°C. Ultimately, cultured DCs were treated with the prepared concentration of 50μg/ml and allowed to incubate for 4 hours. The DCs then went through a series of washing and staining steps with a surface stain for MHC-II first followed by an intracellular stain for TNF-α. The DCs were then fixed with p*araformaldehyde and stored in the dark at 4*°C until flow cytometric analysis.

Results: The data confirmed the hypothesis that wild type MV were indeed able to elicit a notable level of TNF-α production (as an indicator of DC activation) in DCs relative to DCs treated with purified LPS and heat-killed *Salmonella* (HKST) positive controls. The MV treatments were still able to produce strong levels of DC activation over a range of dilutions, while LPS and HKST treatments quickly lost their stimulatory effects after dilution. This indicates that MVs are very potent activators of DCs even at very dilute concentrations.

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Predictors of recurrence in chronic subdural hematoma: do pre-operative, medication, surgical, and other independent risk factors matter?

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Introduction

After burr-hole evacuation of chronic subdural hematoma (CSDH), there is an unacceptably high rate of recurrence: 3-37%. Repeat surgery is necessary, frequently via a larger craniotomy and membrane removal. Predicting which patients may require multiple surgeries is difficult, and there are few data to guide neurosurgeons. Several theories still exist for the cause of CSDH formation, but few explain recurrence after surgery. In this study, we propose to analyze 55 numerical and 40 categorical independent risk predictors analyzing admission, surgical, and post-operative factors which may be associated with recurrent hematoma formation.

Hypothesis

We hypothesize that independent factors such as serum osmolality, hematoma density, and postoperative medications could play a role in the development of chronic subdural hematoma.

Methods

Clinical data on potential predictors of recurrence was abstracted from all consecutive CSDH patients treated at SJRHC in the Bryan/College Station community between 2000 and 2010. Inclusion criteria included all patients treated with surgical intervention using single or multiple burr hole craniostomy under local or general anesthesia followed by closed system drainage. Recurrence of CSDH was defined as an increase in subdural hematoma thickness and volume on the operated side with or without neurological symptoms. Individual chi-square tests and t-tests were used to determine significant predictors of recurrence of CSDH for all numerical values. Equality of Variance tables were used to determine pooled or unpooled degrees of freedom for each predictor, with failure to reject resulting in equal variances. Logistic regression analysis was used to analyze all categorical predictors or recurrence. Significance value was set at $p<0.05$.

Results

A total of 104 CSDH patients were included, with 14 (13.2%) suffering from recurrent CSDH. Post-operative beta blockers ($p=0.033$), incidence of a single number of falls ($p=0.0281$) as well as living situation ($p=0.0013$) were found to be significant predictors of recurrence. Incidence of total falls (single or multiple) showed a trend towards affecting recurrence ($p=0.1043$) but was not deemed statistically significant.

Conclusion

The results of our study indicate a need for the modification of the use of post-operative beta blockers in patients who have undergone craniotomy for chronic subdural hematoma, particularly in elderly patients. Furthermore, all 14 CSDH patients who suffered from recurrence were discharged home, whereas 13 (17%) of nonrecurrent patients were discharged to skilled nursing home. This may indicate a need for longer-term monitoring of patients post-operatively. *Supported in part by the Alpha Omega Alpha Carolyn L. Kuckein Student Research Fellowship*

TAMHSC SUMMER RESEARCH PROGRAM

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Effect of Etifoxine, a Putative TSPO ligand, on the Development of Limbic Epilepsy in the Kindling Model of Epileptogenesis

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Introduction: Neurosteroids are steroids synthesized within the brain from steroid precursors. The biosynthesis of neurosteroids is regulated by the translocator protein (TSPO), which is a five transmembrane domain found in the outer mitochondrial membrane in steroid synthesizing cells. TSPO functions in translocating cholesterol from the outer to the inner mitochondrial membrane. Dysfunction in TSPO and neurosteroid biosynthesis may play a role in neuronal conditions, such as epilepsy. TSPO ligands, such as etifoxine, can enhance neurosteroid synthesis and thereby elicit therapeutic effects. Etifoxine has anxiolytic and neuroprotective properties that can be blocked by the TSPO antagonist PK11195. The main goal of this project is to determine the ability of etifoxine, a putative TSPO ligand, on the development of epileptogenesis, which is the process whereby a normal brain becomes progressively epileptic because of precipitating factors such as injury, fever, and infections.

Hypothesis: It is hypothesized that etifoxine augments neurosteroid biosynthesis in the brain and thereby inhibits or retards the progressive development of limbic epileptogenesis.

Methods: Experiments were conducted in adult mice using a hippocampus kindling model of epileptogenesis. Mice were implanted with a bipolar electrode into the right ventral hippocampus using stereotactic coordinates. The electrode was anchored using three jeweler's screws and dental cement. After a recovery period of 10 days, after-discharge threshold (ADT) was determined by application of 1 s train of 1-ms biphasic rectangular pulses in each mouse. Animals were stimulated daily at 125% of ADT current and behavioral and electrographic seizures were recorded following each stimulation. Etifoxine (0-100 mg/kg, i.p.) was given 30-min prior to stimulations in fully-kindled mice. For developmental studies, etifoxine (25 mg/kg, ip.) was given daily 30-min prior to stimulation. In a second group, PK11195 (2 mg/kg, ip) was given 30-min prior to etifoxine to test for inhibition. Epileptogenesis was evaluated by monitoring the progression of behavioral seizures (Racine scale, stage 0 to 5) and AD duration. The AD was recorded using the Axoscope software.

Results: Etifoxine produced modest antiseizure effects in fully-kindled mice even at the highest dose. Initial results indicate that etifoxine shows trends of inhibition or retardation of development of behavioral and electrographic seizures in mice. Studies are under progress to quantify the kindling retarding effect of etifoxine, and the influence of PK11195 in reversing etifoxine's protective effects on kindling epileptogenesis.

Conclusions: The results of this experiment, although preliminary, show that etifoxine has promising effect on the development of epileptogenesis in the mouse kindling model. Further studies are needed to confirm these pilot studies. ** Supported by NIH grant NS051398 **

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Development of a Basophil Activation Test for Diagnosis of Autoantibodies against FcεR1α

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Introduction

Basophils are granulocytes accounting for less than 1% of circulating blood leukocytes and are implicated in allergic diseases. Basophils can function as effectors of allergic reactions by degranulation release of histamine and as regulatory cells capable of producing cytokines that promote a Th2 immune response. Phenotypically, they are identified by dual expression of the high affinity IgE receptor (FcεR1α) and the IL-3 receptor. Basophil development is dependent on IL-3, and degranulation is mediated by FcεR1α crosslinking, which can be determined by expression of CD63. Autoantibodies against the FcεR1α have been implicated in the pathogenesis of patients with chronic autoimmune urtricaria (CAU). However, reliable assays for diagnosis of CAU are not available. While investigating the functional biology of basophils, **we tested the hypothesis that human basophils synchronized by IL-3 stimulation would serve as a reliable assay for detecting anti-FcεR1α antibodies.**

Specific Aims

1) Investigate the hypothesis that longer duration of priming with IL-3 results in higher sensitivity for human basophil degranulation.
2) Investigate the hypothesis that persons with autoantibodies against FcεR1 have greater amounts of degranulation with longer human basophil exposure to IL-3.
3) Develop a standard curve for FcεR1 crosslinking dependent-basophil degranulation to be used for an *in vitro* diagnostic assay for anti-FcεR1 antibodies.

Methods

Human basophils were isolated from leukopacks by negative selection using Robosep. Basophil purity was measured by flow cytometry using fluorochrome-labeled murine antibodies against IL3-Rα (CD123) and FcεR1α anti-CRA-1). Purity was consistently >98-99% and was confirmed with Wright-Giesma staining. Basophils were incubated with IL-3 (10 ng/mL) for 1 hour, 4 hour, and overnight. The next day, CRA-1 was added at varying concentrations for 30 minutes. Degranulation was measured by flow cytometry detection of CD63 cell surface expression using an anti-CD63 PE mouse antibody.

Results

The kinetics study demonstrated that the optimum human basophil sensitivity for degranulation was overnight incubation with IL-3 and 1 μg/mL CRA-1 exposure. Based on the results of the kinetics study, we were able to establish and validate the ability of our standardized Basophil Activation Test (BAT) to detect anti-FcεR1α (CRA-1) antibodies in serum over a detection range of .1 ng/mL –10μg/mL.

Conclusion

Basophil sensitivity to FcεR1α crosslinking induced degranulation is dependent on prolonged exposure to IL-3. Through our understanding of IL-3 on basophil priming for responsiveness to FcεR1α we have been able to establish a reliable and sensitive bioassay with the potential to diagnose CAU. The BAT has the potential to be developed as an *in vitro* diagnostic assay for antigen specific allergic response. These studies also raise the possibility of targeting IL-3 as therapeutic treatment for basophil-mediated allergic diseases such as CAU.

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Confocal Imaging and Quantification of Neuroprotective Effect of Neurosteroids

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Introduction: Neurosteroids have been recognized as effective anticonvulsant agents and recent work from our laboratory suggests that they may be more effective in treating status epilepticus (SE), a neurological condition characterized by seizures persisting for more than 30 minutes. SE causes profound neuronal damage and hippocampus neurodegeneration, leading to chronic neurological problems in many patients including spontaneous epileptic seizures. There is a strong link between the extent of hippocampal neurodegeneration and the frequency of spontaneous seizures in different animal models and human studies. There is a pressing need for novel therapies for SE with neuroprotective properties to alleviate its long-term impact on neuronal injury. The main purpose of this study is to determine the efficacy of neurosteroid therapy on SE-induced neuronal damage and hippocampal neurodegeneration using confocal imaging and quantification of cell death.

Hypothesis: It is hypothesized that treatment with the neurosteroid THDOC causes significant neuroprotection that is superior to the benzodiazepine diazepam.

Methods: This study utilized brain sections from rats subjected to SE induced by lithium-pilocarpine regimen. THDOC or diazepam was administered 60-min after the onset of SE. Animals were then perfused at 72h after SE induction for immunohistochemical studies. Histological assessments were made by Fluoro-Jade B (FJB) staining of hippocampus subfields CA3, CA1 and dentate hilus regions. FJB is a fluorescein derivative that specifically stains degenerating neurons and therefore allows identification of cell death. Serial images of brain sections were taken using confocal microscopy and the extent of neurodegeration was quantified by counting the number of stained cells in rats with or without neurosteroid treatment using the NIH Image J software.

Results: Preliminary results shows that FJB staining showed extensive neuronal damage in CA1, CA3 pyramidal regions, and dentate hilus regions of the hippocampus in untreated animals, indicating that SE is associated with massive neurodegeneration. In FJB-stained sections, the degenerating neurons in the hippocampus exhibited a bright green fluorescence. THDOC therapy showed significant neuroprotection as evident from reduced number of FJB-positive neurons. Preliminary trends show that the extent of neurodegeneration in animals treated with THDOC was much smaller than in animals treated with diazepam.

Results: The results of this pilot experiment demonstrate the neuroprotective efficacy of the neurosteroid THDOC in the animal model of SE. Further studies are needed to confirm and extend these findings. ** Supported by NIH grant NS071597**

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Influence of the Gut Microbiota Signal Indole on Host Immune Homeostasis & *Salmonella typhimurium* virulence

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Introduction

The human gut is home to a vast and diverse population of non-pathogenic and pathogenic bacteria that contribute to gastrointestinal health and disease. Research suggests that immune responses directed against normal microflora can lead to pathogenesis of inflammatory bowel disease. Additionally, non-pathogenic bacterial signals are capable of deterring pathogenic species and attenuating the host immune response, supporting a mutualistic relationship model between the eukaryotic host and bacterial symbionts. However, the molecular mechanisms behind this paradigm remain largely unknown. Beneath the gut epithelial layer, in the Peyer's Patches (PPs), antigen presenting cells (APCs), such as dendritic cells (DCs), continuously sample and process compounds from the intestinal lumen to instruct the adaptive immune response by cell contact and production of cytokines (TNF-a, IL-10, TGF-B, among others). Naïve T-cells present in the PPs are activated by APCs are induced to differentiate into different T-cell subtypes (Th17, Treg, Th1, Th2, etc).

Hypothesis

Indole, a metabolite of L-tryptophan produced by gut bacteria, acts as a signal to influence bacterial virulence and host inflammatory response. Our hypothesis is a two-fold model where indole attenuates the host-immune response and decreases virulence of pathogens. Specifically, we propose that indole reduces the virulence of the *Salmonella typhimurium* in an *in-vivo* mouse model. In the host DCs, we hypothesize that indole-signaling inhibits inflammatory and promotes anti-inflammatory cytokines through the AhR & NF-KB pathways. Additionally, we propose that indole directs T-cell differentiation to a regulatory anti-inflammatory lineage phenotype.

Methods

Hematopoietic stem cells harvested from C57Bl/6 mice bone marrow were cultured in GM-CSF for 8 days. These bone-marrow derived dendritic cells (BMDCs) were then treated with either indole, solvent, or media overnight and then stimulated with LPS for 4 hrs. The levels of the inflammatory cytokine TNF-a was then measured through flow cytometry on a per cell basis.

C57Bl/6 mice were administered with either indole-treated wild-type *S.typhimurium* or a solvent-treated antibiotic-resistant strain of *S.typhimurium* by oral gavage. At 1 day and 3 days post-infection, mouse organs were harvested and plated on non-selective or enteric-selective agar with or without antibiotics to compare *in vivo* survival of indole- versus solvent-treated *S.typhimirium*.

Mesenteric lymph nodes and spleens were harvested from C57Bl/6 mice and sorted for CD4+/CD25- naïve T-cells by flow cytometry. Sorted naïve T-cells were subjected to Th-17 or T-reg skewing conditions and treated with either indole, solvent or media. The expression of Foxp3, IL-17 or IFN-g by flow cytometry was used to detect T-cell lineage fate on a per cell basis.

To investigate potential pathways of indole signaling in the host, SDS-PAGE was used to detect AhR, CYP1A1, and NF-KB protein levels in intracellular lysates from a dendritic cell line and GM-CSF cultured BMDCs from C57Bl/6 mice.

Results

We show that indole is an important interkindgom-signaling molecule with diverse effects in the GI-tract that supports our two-fold model. Indole inhibits expression of TNF-a in BMDCs and also inhibits *S.typhimirium* growth *in-vivo*. Under T-reg skewing conditions, indole enhances the differentiation of naïve T-cells into the of Foxp3+ regulatory T-cell lineage fate. SDS-PAGE analysis reveals that indole does not affect AhR levels in DCs, despite preliminary evidence of induction of the AhR genetic target CYP1A1. On-going and future studies include SDS PAGE of CYP1A1, and NF-KB levels in DCs after indole treatment, as well as molecular genetic analyses to better elucidate the molecular pathways involved with indole-signaling in the host.

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Transcriptional Control of Mycobacterial Entry Loci (Mel) in *Mycobacterium Tuberculosis*

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Introduction

Mycobacterium tuberculosis (Mtb) is able survive inside host macrophages for extended periods of time, leading to latent infections, amidst microenvironments that expose them to oxidative stress, hypoxia and nutrient deprivation. We have recently shown that mycobacterial entry loci (*mel*) facilitate adherence, entry, and survival of Mtb inside host macrophages. Furthermore, the mel_2 locus has been shown to aid in bacterial resistance to oxidative stress, thus impacting latency. In our current study, we tested the ability of environmental stimuli to regulate expression of *mel* loci.

Hypothesis

There is differential regulation of Mtb loci mel_2 and mel_5 in the presence of fatty acids as a carbon source.

Methods

We conducted Q-PCR analyses of expression of Mel_2 and Mel_5 transcription by growing bacteria under routine laboratory conditions and exposing them to various carbon sources encountered inside macrophages. Functional assays were carried out to observe the impact of a mel_2 deletion on bacterial survival under the same stress conditions. We performed binding assays to evaluate the possibility of an interaction between mel_2 and a unique sigma factor, σ^B, involved in transcription.

Results

We demonstrated that mel_2 is likely regulated via the sigma factor σ^B and that, by using reporter assays, the mel_2 promoter is active under nutrient-limiting and propionate growth conditions. Q-PCR revealed a quantitative induction of mel_2 transcripts in the presence of propionate as the sole carbon source. Data also showed that mel_2 is monocistronic and transcribed as one unit consisting of six genes (mel_{F-K}). It is likely that mel_2 plays a role in propionate metabolism. Further studies to confirm this hypothesis are in progress. In summary, we have shown that mel_2 plays a role in fatty acid metabolism, a primary carbon source for mycobacteria inside the host.

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Small Molecule Inhibitor WP1130 Downregulates Mcl-1 via USP9x Inhibition and Sensitizes to Bcl-XL Inhibitor ABT-737 in Lung and Colon Carcinoma

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Introduction

In many tumors it has been shown that overexpression of pro-survival Bcl-2 family members Bcl-XL and Mcl-1, confers resistance to a variety of chemotherapeutic agents. Mcl-1 is a critical protein for survival for a variety of cell lineages and is a unique member of the Bcl-2 family of proteins in that its short half-life is mediated by its rapid turnover via ubiquitination. In many tumors, it has been shown that deubuitinases (DUB) are overexpressed and activated and may contribute to the cancer phenotype. Of note, deubiquitinase USP9x serves to regulate Mcl-1 and prevent its ubquitination, thereby preserving its pro-survival function. Small molecule WP1130 acts as a partially selective deubiquitinase inhibitor - targeting USP9x, USP5, USP14, and UCH37 all of which are known to regulate survival protein stability and regulate proteasome function. Using WP1130 to inhibit Mcl-1 could sensitize cells to Bcl-XL inhibition and result in a powerful new treatment strategy.

Hypothesis

Downregulation of Mcl-1 via USP9x inhibition sensitizes tumors to Bcl-XL inhibition and results in apoptosis.

Methods

Tissue array staining was completed using immunohistochemistry to demonstrate Bcl-XL, Mcl-1, and USP9x overexpression in lung and colon cancers. Western blots were used to examine protein levels at baseline and after treatments. Cell death was quantified using Annexin V and flow cytometry. RNA transfections were carried out using Lipofectamine RNAMAX and cDNA transfections were carried out using LIpofectamine 2000. Cytotoxicity was assessed using XTT method. ABT-737, SAHA, cisplatin, and WP1130 were purchased from commercial providers.

Results

We show that Bcl-XL and Mcl-1 are overexpressed in colon and lung cancer and are correlated with stage in colon cancer. Our results indicate that use of WP1130 inhibits USP9x leads to Mcl-1 degradation and apoptosis in a variety of tumor environments. Additionally, we demonstrate that siRNA against Mcl-1 and Bcl-XL together result in poor tumor survival and consequently, use of WP1130 with ABT-737, a well-documented Bcl-XL inhibitor, demonstrates chemotherapeutic synergy in a variety of tumor lineages, suggesting this approach as a novel treatment paradigm.

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Modulation of TGF-beta-induced Apoptosis of Ascending Aortic Vascular Smooth Muscle by Sex Steroids and Relationship to Dissection of Thoracic Aortic Aneurysms

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Introduction

Thoracic aortic aneurisms (TAAs) are the 13th leading cause of death in the United States and are a primary cause of death in people with connective tissue associated genetic disorders such as Marfan's Syndrome, Ehlers-Danlos Syndrome Type IV, and Loeys-Dietz Syndrome. Aneurism formation results from a thinning of the vessel wall, which is due to the degeneration of extra cellular matrix and apoptosis of vascular smooth muscle cells; both of which have been highly correlated with increased activity of the cellular messenger TGF-beta in ascending aortic vascular smooth muscle cells (AAVSMCs.) Interestingly, epidemiological studies have shown sexual dimorphism regarding the morbidity and mortality of TAAs in humans; and the same phenomenon has also been observed in our low fibrillin-1 expressing mouse models, where in both cases, the pathological timeline for males is more severe. Because this trend has yet to be investigated as it pertains to TGF-beta, the purpose of this experiment is to examine the effect of androgen and estrogen on TGF-beta mediated vascular pathology: specifically, ascending aortic vascular smooth muscle cell apoptosis. We hypothesize that androgen treatment will increase apoptosis in cultured TGF-beta treated AAVSMCs and that estrogen treatment will decrease apoptosis in cultured TGF-beta treated AAVSMCs.

Methods

To test these hypotheses, we first determined the concentration of TGF-beta necessary to induce apoptosis in the AAVSMCs using a tunnel assay and by doing qRT-PCR for caspase8, caspase9, and bcl2. We next looked at the effects of methyltrienolone and 17-beta-estradiol using the same assays. We found that the TGF-beta treatments did have an effect on the viability of AAVSMCs, but additional experimentation is underway to ascertain the role of methyltrienolone and 17-beta-estradiol.

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Membrane Vesicles of *S. typhi* As Targets for the Development of Innovative Vaccines

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Introduction

As pathogens are able to become multi-drug resistant, the development of vaccines against infectious organisms by using different approaches is necessary. Membrane vesicles (MVs) are lipid and protein nano-particles derived from bacterial periplasm and outer membranes of Gram-negative bacteria. They contain compounds that are recognized by host microbial pattern recognition receptors as well as surface expressed antigens. These can be recognized by pathogen-specific lymphocytes.

This research intends to develop rapid and inexpensive treatment and prevention strategies against infectious diseases caused by Category A and B facultative intracellular Gram-negative bacterial pathogens. The membrane vesicles to investigate are from an attenuated mutant strain of *Salmollela typhi*. This strain has a deletion in the *aroC*1019 gene and requires 2,3 dihydroxybenzoic acid, an iron uptaker, for survival.

Hypothesis

The central hypothesis of this research plan is that MVs can be easily isolated and highly manipulable pro-inflammatory and poly-antigenic, nano-particle facsimiles of pathogenic bacteria that possess novel biophysical properties, combining to induce robust protective immunity. The overall goals are to evaluate the suitability of the MV platform for establishing vaccine-induced immunity to a broad-spectrum of bacterial pathogens. In this project, we focus on MVs of the mutant strain of *S. Typhi*.

Methods

The research design is broken down into two specific aims as follows:

1. Testing the hypothesis that *Ty*MVs have important and malleable biophysical and adjuvant properties for dendritic cell (DC) activation. This aim is further broken down into 3 experimental approaches: establishing culture conditions for maximal production of MVs, evaluating the biophysical properties of *Ty*MVs, and determining the DC stimulation properties of *Ty*MVs as a measure of adjuvant activity and vaccine potential.
2. Determining the correlates of protective immunity for *Ty*MV immunization. This aim is further broken down into 2 experimental approaches: establishing optimal MV immunization conditions and regimens that induce maximum *S. typhi* specific immunity, and challenging *Ty*MV immune mice to determine protection from infection.

Results

The results to obtain by the end of the project are as follows:

1. Methods for maximal production of MVs
2. Total protein content and dry weights of each MV batch
3. Protein content by SDS-PAGE
4. DC stimulatory properties
5. Immunization induced antibody responses

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Role of Fbw7, a βc Ubiquitin Ligase, in IL-5 Receptor Function

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Introduction

IL-5 is a secretory product of CD4 + TH2 lymphocytes whose target cell is the eosinophil. Aberrant activation of eosinophils by IL-5 underlies some of the pathology seen in several inflammatory disorders such as allergic asthma, some lymphomas and hypereosinophilic syndrome. The IL-5 receptor (IL-5R) consists of a ligand-binding α chain and a common signaling beta chain, βc, that is shared by two related cytokines, IL-3 and GM-CSF. Binding of IL-5 to its receptor leads to both βc tyrosine phosphorylation and ubiquitination. Findings from our laboratory have demonstrated that inhibiting βc ubiquitination results in dampened IL-5-induced signaling, indicating that targeting this process might provide an alternative therapeutic intervention for treating eosinophilic inflammatory disorders. Although much is known about activation of IL-5 signal transduction, the molecular mechanisms underlying βc ubiquitination are currently unknown. Recently, a bioinformatics analysis of the βc cytoplasmic domain identified three potential ubiquitin ligase binding sites: Fbw7, β-TrCP, and TRAF2. Moreover, our preliminary studies show reduced βc ubiquitination in individual receptor mutants defective in Ub ligase binding, confirming their role in this process. Therefore, our goal in this study is to characterize the role of one βc Ub ligase, Fbw7, in IL-5R biology.

Hypothesis

Since Fbw7 binds to and contributes to βc ubiquitination, we predict that depleting its expression by RNAi will alter IL-5R function and dampen signaling.

Methods

RNAi (lentiviral shRNAs) were used to deplete Fbw7 protein levels in the TF1-F11 cell line. Biochemical techniques were used to evaluate βc and 5Rα protein stability.

Results

Combinatorial RNAi with two different shRNA constructs directed at Fbw7 in concurrence with individual utilization of each construct demonstrated efficient reduction of Fbw7 protein levels in TF1 cells. Accumulation of βc and IL-5Rα proteins were observed in TF1 cells expressing decreased Fbw7 protein levels. The data suggest that Fbw7 might play a role in regulating βc and IL-5Rα protein stability.

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Distinguishing Esophageal and Endotracheal Intubation by Measuring Cuff Pressure

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Introduction

Inadvertent esophageal intubation leads to serious and potentially lethal complications when undetected. Whether measuring cuff pressure (CP) can accurately differentiate esophageal from tracheal intubation is unknown. Studies using a live porcine model found a difference in CP between the esophagus and the trachea but a human cadaveric study did not support this finding. This study was designed to resolve this conflict using live human subjects to determine whether proper placement of the endotracheal tube could be confirmed through measurement of CP.

Hypothesis

Due to structural differences between the esophagus and trachea, we expect CP to initially be higher in the esophagus, but to increase more rapidly in the trachea as the cuff is inflated against the cartilaginous rings of the airway. At high cuff volumes, tracheal CP should exceed esophageal CP.

Methods

34 female and 35 male patients undergoing elective surgery were intubated with 7.0 and 8.0 mm cuffed tubes, respectively, both esophageally then tracheally. CP was recorded after each one mL of inflation until a volume of 10 mL was reached or CP exceeded 250 mmHg. Esophageal and tracheal CP were compared using the Wilcoxon signed rank test.

Results

In females, tracheal CP was significantly higher than esophageal CP at volumes between 3 and 10 mL ($p<0.05$). The pressure difference was greatest at 9 mL, where tracheal CP averaged 70 mmHg higher (SD 63 mmHg, $p<0.0001$) than the esophageal CP. In contrast, in males, esophageal CP was significantly higher at volumes less than 4 mL. The pressure difference (10 mmHg, SD 48 mmHg, p 0.15) was greatest at 7 mL, though this did not reach statistical significance.

Conclusions

At greater cuff volumes, tracheal CP was found to be higher than esophageal CP in females but not in males. However, the high degree of variability between patients limits the clinical usefulness of this measurement as a method to confirm proper placement of an endotracheal tube.

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Anatomical and Physiological Assessment of *Callithrix jacchus* in Preparation for Invasive Neurophysiology

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Introduction: People suffering from age-related hearing loss (presbycusis) have difficulty listening in environments where multiple competing sounds are present (cocktail-party situations). Whereas listeners with normal hearing can separate sounds and attend only to a sound of interest, in presbycusis the sounds fuse and the listener is unable to separate them. Selective listening is a fundamental and behaviorally important sensory capability exhibited by most vertebrates. In humans it is important in social interactions. In animals in the wild, it is often necessary for survival.

Directional hearing (DH) is crucial for sound separation because knowing the location of a sound source allows the listener to selectively listen in that direction while suppressing sounds originating from other directions. DH is impaired in presbycusis although the specific neural deficits are largely unknown. It is known that the lateral superior olive (LSO) specializes in DH, using interaural intensity differences (IIDs) to compute the location of sounds. Thus, a loss in DH with age may result from functional deficits in the LSO.

We address these questions in a non-human primate model (the common marmoset, *Callithrix jacchus*), which is fast-maturing and hence useful for studying the effects of aging. Although the behavioral aspects of the problem have been researched intensively, very little is known about the neural mechanisms underlying the cocktail-party effect or why these mechanisms fail in presbycusis. Further, most of the work has taken place in model systems that are unrelated to primates and therefore, cannot be readily translated to human hearing.

Specific Aims and Implications: Our immediate goal is to establish a non-human primate as a model for studying behavioral and neurophysiological deficits that result from aging. This goal will be achieved in 3 steps (outlined in methods): 1) Behavioral testing and training. 2) Identify candidates. 3) Prototype recording procedures.

Methods: 1) Behavioral testing and training: Marmosets will be trained to respond to different auditory stimuli, providing an easy way of identifying patients with hearing loss. 2) Identify candidates: Chronically implanted electrodes will be inserted into both Lateral Superior Olive (LSO) Nuclei. Post recovery, evoked stimuli will be recorded from each LSO every two weeks. 3) Prototyping recording procedures: Recording and data collecting procedures will be systematic and repeatable. Recordings will be taken from the auditory nerve root, the inferior colliculi, the cochlear nucleus, and the LSO.

Data Analysis: The average amplitude of evoked activity of LSO (Aim 2) will be the response measures. The responses will be plotted as a function of the noise IID using the analysis method outlined in Ratnam & Feng [1998]. If directional hearing is not impaired then the response strength will increase as the IID for N pulls it away spatially away (virtually) from T. That is, noise is less effective in masking a neuron's response as the angular separation is increased. We expect to see this in young controls. In older subjects we expect to see reduced improvement in response as N is moved away from T. We will compare the differences in the response vs. IID curves for old and young subjects and assess the differences using a *t-test*.

Results: With step one well underway and step three completed, we believe we have found the non-human primate model for studying age related hearing loss. The lab has developed a systematic method for developing gold plated platinum iridium wire tetrodes that will be used for the recordings, and has perfected auditory stimulation protocols and methods for data collection. We are now ready for the invasive neurophysiology and chronic implantation of electrodes. These will be used to monitor changes in neuronal signaling in response to auditory stimuli as each marmoset ages.

Regulation of the Cyclin-Dependent Kinases during Human Cytomegalovirus Lytic Infection and Their Role in Virus Reactivation

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Human cytomegalovirus (HCMV) is a member of the herpesvirus family. HCMV infection is largely asymptomatic, but as an opportunistic pathogen, significant clinical manifestations of infection are observed in immunocompromised individuals and newborns. In the United States, HCMV infects 50-80% of the population by age 40; moreover, the virus remains in a latent state in the host after primary infection. The primary reservoirs of the virus in the body are cells of the myeloid lineage, and the virus is reactivated upon differentiation of monocytes into macrophages.

It has been previously established in fibroblasts that HCMV infection affects the accumulation of proteins involved in cell cycle regulation. HCMV induces accumulation of the G_1-phase cyclin E and M-phase cyclin B, resulting in a concomitant increase in their associated kinase activities. At the same time, expression of the S-phase cyclin A is suppressed. In addition, HCMV infection causes accumulation of the tumor suppressor p53; however, the cdk inhibitory protein, p21, a downstream target of p53, does not increase in parallel. The expression of immediate-early (IE) viral genes is also dependent on the phase of the cell cycle at the time of infection. Cells infected in G_0/G_1 express IE genes immediately after infection. In contrast, cells infected during S-phase exhibit a block to IE gene expression until the cells transition back into G_1. This S-phase block to IE gene expression can be bypassed by treating cells with the cdk inhibitor SU 9516, which targets cdk-2, -1, and -4. It has also been reported that SU 9516 can induce reactivation of the virus in NT2 cells, which serve as a model for viral latency. These results are surprising result given the requirement for cdk activity during the early and late phase of lytic infection of primary cells.

THP-1 monocytes are conditionally permissive for viral infection and support replication of the virus upon differentiation into macrophage-like cells. The effects of HCMV infection on the expression of cell cycle regulatory proteins in primary or THP-1 derived macrophages have not been determined. We investigated the expression of cyclins, cdks, and p21 in THP-1 derived macrophages. We found that HCMV infection of p53-negative THP-1 macrophages causes accumulation of cyclins B and E and inhibits cyclin A, suggesting that p53 is not involved in the regulation of these proteins. Because of the recent findings showing that the cdk inhibitor SU 9516 can induce reactivation (IE gene expression) in NT2 cells, we tested whether the same was true in THP-1 cells, which serve as a more relevant model for HCMV latency. We tested the abilities of three cdk inhibitors with differing specificities to reactivate IE gene expression in latently infected THP-1 monocytes. Our preliminary results indicate that a reduction of cdk1 activity during the process of differentiation is not sufficient for reactivation of viral gene expression.

2011 College of Medicine
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Roy Jones	Dr. Robert Alaniz
Kent Cochran	Dr. Gregory Bix
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William Samuelson	Dr. Emily Wilson
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Jae Cho	Dr. Sarah Bondos
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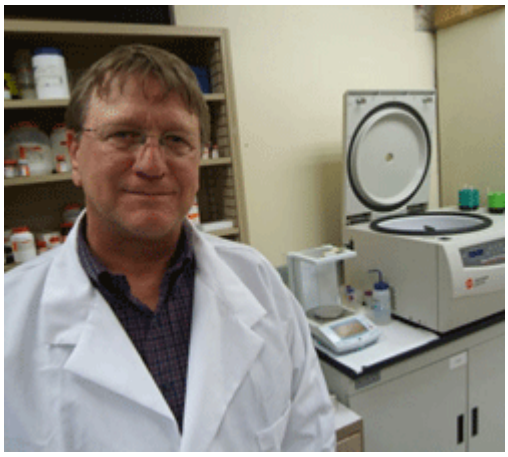
Sarah Ho	Dr. Magnus Hook
Soumili Chatterjee	Dr. Steve Safe & Dr. David Huston
Tyler Buchanan	Dr. Magnus Hook

2011 College of Medicine
Summer Research Program Seminar Series

Date	Time	Topic	Presenter
6/7	12:00 PM	Record Keeping	Dr. Van Wilson
6/10	9:00 AM	Scientific Method	Dr. David McMurray
6/14	12:00 PM	Animal Research	Dr. Helene Andrews-Polymenis
6/17	9:00 AM	MMPA	Dr. James Samuel
6/21	12:00 PM	Scientific Misconduct	Dr. Vernon Tesh
6/24	9:00 AM	Biotechnology Careers: Commercialization in Science & Medicine	Mr. Bruce Leander, Retired President, Ambion, Inc.
6/28	12:00 PM	MD/PhD Program	Dr. Julian Leibowitz & MD/PhD students
7/1	9:00 AM	Molecular Pathogenesis	Dr. Jeffrey D. Cirillo
7/5	12:00 PM	Medical Research…Why Me?	Dr. W.C. Culp, Jr.
7/8	9:00 AM	Biotechnology/Ethics	Dr. James Samuel
7/12	12:00 PM	Neuroscience	Dr. Rajesh Miranda
7/15	9:00 AM	Clinical Medicine	Dr. David Huston
7/19	12:00 PM	Dysregulation of Biogenic Amine Metabolis in Biliary Cancer	Dr. Sharon DeMorrow
7/22	9:00 AM	Human Experimentation	Dr. John Quarles
7/26	12:00 PM	Graduate Studies	Dr. Emily Wilson
7/29	9:00 AM	Cell and Molecular Biology Biochemistry and Structural Biology	Dr. Kayla Bayless Dr. Sarah Bondos
8/2	12:00 PM	Student Oral Presentations	
8/5	9:00 AM	Student Oral Presentations	
8/9	12:00 PM	Student Oral Presentations	
8/12	9:00 AM	Student Oral Presentations	
8/17	9:00AM-2:00PM	Poster Presentations and Reception	



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