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REGULAR PHYSICAL ACTIVITY REDUCES CHRONIC ALCOHOL-INDUCED HEPATIC INJURY IN RATS

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Many individuals throughout the world perform regular aerobic exercises and consume alcoholic beverages, which raises the question: what would be the interactive effects of exercise and ethanol on the hepatic oxidant/antioxidant system? Our project tested the hypothesis that physical conditioning abrogates the hepatic oxidative injury caused by chronic alcohol ingestion in rats. Male Fisher rats were divided into three groups of seven animals each and treated as follows: 1) control rats orally fed 5% sucrose daily for 12 weeks; 2) rats orally fed ethanol daily at a dose of 4 g/kg for 12 weeks; and 3) rats given exercise training on treadmill and fed ethanol daily at a dose of 4 g/kg for 12 weeks. Body weight and blood pressure (BP) was recorded weekly through tail-cuff method. At the end of the treatment, rats were sacrificed; blood and liver were isolated and analyzed for ethanol concentration and oxidative stress parameters.

Chronic alcohol administration caused no significant change in body weight but increased mean blood pressure and blood ethanol concentration compared to rats in the control group. Exercise training significantly lowered the body weight and changes in BP and blood ethanol concentration that were caused by ethanol treatment. Chronic ethanol treatment significantly increased hepatic malondialdehyde (MDA) levels, NADPH oxidase and Mn-superoxide dismutase activities and depressed enzyme activities of CuZn-superoxide dismutase, catalase and glutathione peroxidase in rats. Exercise training for 12 weeks significantly ameliorated the changes in the hepatic MDA levels, NADPH oxidase, and antioxidant enzyme activities caused by chronic alcohol treatment in rats.

The study concluded that regular aerobic exercise activity ameliorates the hepatic oxidative injury caused by chronic ethanol ingestion in rats by lowering the body weight, blood ethanol concentration and enhancing the hepatic antioxidant defense system.

IMPACT OF CARPAL TUNNEL RELEASE (CTR) ON DIABETICS VS NON-DIABETICS

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For those with carpal tunnel syndrome (CTS), clinical relief of hand numbness and tingling varies among diabetics and non-diabetics who receive carpal tunnel release surgery. In addition, post-operative wound healing character and pain appears to differ between these groups. Carpal tunnel release (CTR) surgery is performed in an effort to relieve numbness in the fingers and palm region. All patients who underwent carpal tunnel release surgery were surveyed to determine different experiences between those who were diabetic and non-diabetics.

Patients with CTS were interviewed after surgery in order to gain a better understanding of the nature of their conditions. A survey was then mailed to all patients of a selected physician to assess the post-operative results of CTR surgery. Items on the questionnaire asked the patient to identify the feeling and sensation of the hand, wrist, and fingers after CTR surgery. The questions were designed to isolate responses for diabetic and non-diabetic patients and to measure varying conditions during different times of the day. Questions specific to diabetic patients assessed the impact of their diabetes treatment on the carpal tunnel condition.

Results are pending.

TESTOSTERONE EFFECTS IN STIMULATING CARDIOMYOCYTE TRANSCRIPTION FACTOR EXPRESSION IN MURINE EMBRYONIC STEM CELL DIFFERENTIATION

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Embryonic stem cells (ES cells) have been shown to hold great potential for therapeutic regenerative medicine, eg, in healing heart muscle tissue after coronary infarction. The ES cells are totipotent, since they have the capability to differentiate into cells of every organ in the body, including tissues that have very little renewal capacity, in particular those found in the heart. Murine ES cells can be propagated indefinitely on a feeder-layer of mitotically inactivated mouse embryonic fibroblast (MEF) cells and then allowed to differentiate by mass culture into embryoid bodies (EB), forming a large variety of cells, including colonies of beating cardiomyocytes. Recently, our laboratory has confirmed preliminary work showing that embryoid bodies treated with testosterone are stimulated to increase cardiomyocyte differentiation by about three-fold. We hypothesized that testosterone treatment of EB cultures causes an increase in the expression of key cardiac transcription factors Nkx2.5 and MEF2C as measured by RT-PCR. We grew ES cell cultures for 5 to 18 days with or without 100 nM testosterone, and observed that some colonies of cells spontaneously contracted rhythmically like cardiomyocytes. Cultures were harvested for purification of total RNA and protein extracts, and the mRNA was subjected to real-time RT-PCR for analysis of gene expression levels for transcription factors MEF2C and Nkx2.5, and also the later cardiac markers myosin light chain 2V and myocardin. We observed about two-fold stimulation of MEF2C and Nkx2.5 by testosterone, consistent with our hypothesis. However it is still unknown if treating the ES cells with testosterone earlier on 2 days of growth will cause a larger increase in the quantity of cardiomyocytes. In future work, we plan to test if earlier treatment will cause an increase in the quantity of differentiated cardiomyocytes. In our past work, we have shown that testosterone increases the differentiation of mesenchymal stem cells into skeletal myocytes. By the stimulation of cardiomyocyte differentiation from embryonic stem cells, we hope to improve methods of ES cell determination for cardiac therapy eventually for human patients.

AN ANALYSIS OF HEART TISSUE FOR MRNA LEVELS FOR SELENOPROTEIN FAMILY MEMBERS

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Selenium (Se) is a dietary trace element essential for a various aspects of human health including metabolism, immune responses, and cancer prevention. Mounting evidence exists that increased levels of Se intake may protect against cardiac diseases. For example, Se supplementation has been shown to protect against cardiac damage occurring from ischemia-reperfusion injury, adriamycin-induced cardiotoxicity, and diabetes-induced cardiopathology. However, the mechanism by which Se supplementation carries out its protective effects remains unclear.

Se exerts its biological effects through its direct incorporation into selenoproteins as the amino acid, selenocysteine. To date, 25 selenoproteins have been identified in humans. Several selenoproteins have been shown to play important roles as antioxidant enzymes, while the functions of many have yet to be identified. More specifically, exactly which of the selenoproteins are expressed in heart tissue and how that expression is regulated are poorly understood. Therefore, a pilot study was conducted in order to determine which selenoproteins are expressed in mouse heart tissue at the level of mRNA. Using real-time quantitative PCR (qPCR), mRNA abundance was determined for each selenoprotein, as well as important factors involved in selenoprotein synthesis; the results are pending.

PREDICTORS AND OUTCOMES OF ABNORMAL PULMONARY TESTS IN SUBJECTS AWAITING LIVER TRANSPLANTATION

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Pulmonary changes are common among patients with cirrhosis. The impact of these changes on the health outcome of patients who were on liver transplantation (LT) list is not well-characterized. The objectives of this study were 1) to describe the prevalence of abnormal (A-a) gradient and airway obstruction in patients who were on LT list; 2) to determine the predictors of abnormal (A-a) gradient and airway obstruction, and 3) to determine impact of abnormal (A-a) gradient and airway obstruction on survival outcome while subjects were on LT list.

All subjects who were on LT list at the Indiana University from Jan 1999–July 2006 were studied. Demographic data, pulmonary tests and arterial blood gas profiles were collected. Patients were followed until death, transplantation, or study closure date (July 30, 2006). Abnormal (A-a) gradient was calculated based on previously reported formula. Abnormal airway obstruction was defined according to the American Thoracic Society guidelines. Univariate and multivariate logistic regression were performed to identify variables associated with abnormal (A-a) gradient and airway obstruction. Survival analyses were assessed with Kaplan-Meier estimates.

A total of 417 subjects were included. Abnormal (A-a) gradient and airway obstruction were found in 73% and 54% of subjects, respectively. Twenty five patients died and 346 subjects underwent LT. Independent predictors of abnormal (A-a) gradient were smoking (OR 1.56), high BMI (OR 1.08), abnormal chest x-ray (OR 3.24), and hypertension (OR 1.56). Smoking (OR 2.75) was the only predictor of airway obstruction. Subjects with abnormal (A-a) gradient had shorter median survival (1,181 days) when compared to those with normal gradient (1,456 days, $P=.03$).

Gas exchange abnormalities and airway obstruction are common in cirrhotic patients awaiting liver transplantation. Smoking was an independent predictor for both abnormal gradient and airway obstruction. Abnormal (A-a) gradient was a predictor of death while on LT list. Smoking cessation and optimization of abnormal gas exchange should be incorporated in the pre LT care.

RETENTION OF DIABETES EDUCATION AMONG DIABETES CLINIC PATIENTS AND RELATION WITH DIABETES CONTROL IN TERMS OF HBA1C, BLOOD PRESSURE AND CHOLESTEROL

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Objective. We are planning to study the retention of diabetes education among clinic patients at different intervals of time and in relation with diabetes control as measured by HbA1C, blood pressure and cholesterol levels.

Design. This testing was performed to study the retention and effectiveness of diabetes education delivered by trained diabetes educators during a three-hour session in the clinic. The purpose of the study was explained to each patient enrolled and informed consent was obtained. A five question pre- and post-test was administered to each patient who attended the diabetes education class. The test consisted of five questions, designed to determine the patient's knowledge of goals for HbA1C, blood pressure and cholesterol. In our study, each patient enrolled was administered the same test (retention test) at least three months after the class. The scores were compared with the previous tests. Retainers were defined as patients whose score was the same or higher than when the test was administered immediately after the class. Non-retainers was defined as patients whose score in the retention test was lower than their scores in the post-test. HbA1C, blood pressure and cholesterol levels measured within one month before and after of the diabetes education class were compared with measurements taken within one month before or after the retention test. Fifty subjects participated in this study. Results are pending.

CHARACTERIZATION OF BLOOD DERIVED STEM CELLS

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Non-embryonic stem cells continue to be of interest in providing an alternate source of pluripotent progenitor cells. However, little is known regarding the resiliency, longevity and the ability to continuously revert to stem cell phenotype of select non-embryonic stem cell populations. We investigated the ability of blood monocyte-derived stem cells to maintain their plasticity in culture at differing dosing regimens, culture intervals, and repeated growth factor challenge. Blood monocytes were isolated by density centrifugation (Ficoll) with subsequent positive selection and converted to macrophages (adherent cells post-culture confirmed by flow cytometry, CD14+). Macrophages, in the presence or absence of non-adherent cells and/or their culture medium, reverted to fibroblastic-like macrophage (F-macs, CD34+) stem cells after culture with growth factors, M-CSF and LIF. F-macs were cultured in the absence of growth factors with subsequent addition of growth factors after starvation intervals, and morphology, viability (trypan blue exclusion dye), and the ability to maintain or revert to F-mac status was determined. Propagation and long-term storage potential of F-macs were assessed.

END OF MUCOIDY: THE ROLE OF RpoN IN PROMOTING ALGINATE PRODUCTION IN *PSEUDOMONAS AERUGINOSA*

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Mentor: SusanH Jackman, PhD, Joan C. Edwards School of Medicine, Huntington, West Virginia

According to a recent survey by the Cystic Fibrosis (CF) Foundation, 30,000 Americans live with CF, a debilitating genetic disease. Often times, these individuals die from severe pneumonia caused by a bacterium called *Pseudomonas aeruginosa*. This ubiquitous bacterium infects the patient's lungs and converts from a nonmucoid to mucoid form by producing a polysaccharide called alginate. Several regulators of alginate have been identified, including two sigma factors called AlgU and RpoN. However, the exact nature of RpoN's involvement is elusive. The objective of this project was to use recombineering technology to knockout the RpoN gene in *P. aeruginosa* to examine the effect of RpoN on mucoidy. To achieve this, a DNA fragment encompassing RpoN was amplified using the polymerase chain reaction (PCR). The PCR product was cloned into a *P. aeruginosa* suicide vector called pEX100 and introduced into an *E. coli* strain carrying a phage lambda recombination system called Red. A second PCR product was made using a set of extra long hybrid primers homologous to RpoN and the template plasmid flanked by a gentamicin resistance cassette. When the Red system was induced at 42 C, the added PCR product was allowed to insert into RpoN on pEX100 via homologous recombination, creating a new plasmid containing inactivated RpoN. This plasmid was conjugated into *P. aeruginosa* PAO579 for gene replacement. The PAO579 mutant was nonmucoid. Also, when RpoN was reintroduced on a plasmid into the RpoN mutant through conjugation, mucoidy was restored, thus proving that RpoN is required for mucoidy in *P. aeruginosa* PAO579.

MECHANISMS OF AEROALLERGEN SENSITIZATION IN MICE WITH EOSINOPHILIC ESOPHAGITIS

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Background. Eosinophilic esophagitis (EE) is an inflammatory disease of the esophagus caused by multiple food allergies. It is mainly characterized by eosinophilic infiltration of the esophagus. Patients with EE often have asthma and allergic rhinitis. An experimental model of EE in mice was generated using a large volume of aeroallergen, suggesting that allergen sensitization occurs through the lungs.

Objective. Our goal was to determine whether allergen sensitization occurs via nasal passages as well.

Methods. Mice were primed with two intraperitoneal treatments of Aspergillus fumigatus, then challenged with three intranasal treatments of either 5 uL or 50 uL of *A. fumigatus* (nasal group and lung group, respectively). Their respective control groups were equally treated with saline solution. Mice were sacrificed within 24 hours of the last treatment. The esophagus, lung, and spleen were harvested. Immunohistochemical staining for eosinophils was performed on lung and esophageal sections. In vitro spleen lymphocyte culture in the presence of *A. fumigatus* was performed; supernatants were assayed for the levels of the secreted cytokines IL-4, IL-5, IL-13 and IFN. Comparison between allergen-challenged mice and controls was done with respect to the number of esophageal and lung eosinophils, and the levels of cytokines produced. Comparisons with respect to these parameters were also made between the nasal and lung groups.

Results. The number of esophageal eosinophils was similar between the nasal and lung groups. Both groups of mice had higher Th2 cytokines than their respective controls, indicating an allergic phenomenon.

Conclusion. EE may be induced in mice via allergen sensitization in either the lungs or nasal passages.

XPD POLYMORPHISMS AND NON-MELANOMA SKIN CANCER RISK IN THE PUERTO RICAN POPULATION

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Non-melanoma skin cancers (NMSC) which are categorized as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are the most common cancer in the western world. Risk factors for NMSC's include excessive exposure to UV, skin type, age, and a low DNA repair capacity (DRC). Mutations in tumor suppressor genes, cell growth regulator and nucleotide excision repair (NER) genes can also contribute to the development of BCC's. The XPD gene is a DNA repair gene that functions as a helicase in the NER mechanism and as a transcription factor in the cell-cycle. In this study, we tested the hypothesis that a mutation at the XPD gene in position 751 (Lys751Gln) is a risk factor for developing BCC at early age ($\leq$35 years). Our objectives were to: 1) determine the frequency of the XPD allele in the Puerto Rican population without skin cancer; 2) evaluate the presence of the mutant allele as a risk factor for developing early onset BCC; and 3) compare the DRC of the young BCC's patients with the age-matched controls. For testing our hypothesis, we used DNA from peripheral lymphocytes, PCR, *Pst I*, genotyping (AA, AC, CC) and statistical analyses. The presence of the XPD mutant allele showed an OR=7.0 for the development of BCC at an early age. In addition, the DRC in BCC patients showed a statistically significant reduction of 47% (P=0.037), when compared to age-matched controls. This study included an under-studied population and may provide insights about genetic predisposition to developing BCC.

SELENIOUS ACID ENHANCES MULTIDRUG RESISTANCE [MDR1] MRNA EXPRESSION IN PROSTATE CANCER

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Nearly 50% of human cancers are either completely resistant to chemotherapy or only respond momentarily, after which they are no longer affected by commonly used anticancer drugs. This phenomenon is referred to as multidrug resistance and is inherently expressed by some tumor types while others acquire multidrug resistance after exposure to chemotherapy treatment. MDR1 encodes an energy-dependent drug efflux pump that is responsible for decreased drug accumulation in multi-drug-resistant cells. Increased expression of MDR1 in cancer cells is common; however, in prostate cancer, decreased expression in MDR1 is linked to disease progression. In our lab, a promoter of MDR1 gene has been shown to be hypermethylated (hydrogen atom [H] is replaced with a methyl group [CH3] regardless of substrate). When hypermethylation occurs, the gene is silenced downstream. For this project, we will treat prostate cancer cell lines with Selenious acid, a non metal chemical element used in prostate cancer prevention, and control demethylation agent 5-azacytidine (AZC), a DNA methylation inhibitor. We hypothesize that the treatment of prostate cancer cell lines with selenium will lead to the demethylation of MDR1 promoter region and subsequently increase in gene expression.

IDENTIFICATION OF RESET-FACILITATING BINDING COMPLEX OF THE IRS-1 PROTEIN IN RAT HEPATOMA CELLS

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Pre-diabetes is a pathophysiological condition in which cells become resistant to the insulin signal, which is instrumental to the absorption and metabolism of glucose. One important component of the subcellular insulin signaling pathway involves IRS protein phosphorylation near the cell membrane and translocation to propagate the insulin signal. However, it must then dephosphorylate and relocate to the cell membrane to reset the pathway. One possible means by which this could be accomplished is the formation of a protein-protein complex. Identification of IRS-associating reset-specific binding proteins could provide a possible link between phosphorylation reduction and insulin resistance, aiding our understanding of the pre-diabetes condition and the development of new treatments.

It was hypothesized that binding proteins, including Ser/Thr phosphatases, mediate IRS-1 membrane reassociation and dephosphorylation. To this end, H4IIE rat hepatoma cells expressing FLAG-tagged human IRS-1 were treated with insulin for 20 minutes (representing the insulin-stimulated pathway) or treated with insulin for 20 minutes followed by a 2 hour wash-out (representing the pathway reset/ dephosphorylation and relocation of the IRS-1) against baselines in only HCl carrier solution. Cells were then lysed and IRS-1 (and any bound proteins) were coimmunoprecipitated with αFLAG-agarose. Proteins were then eluted using FLAG peptide.

The resulting stock solutions were: 1) assayed to determine protein concentration; 2) ran through SDS_PAGE and immunoblotting to confirm equivalent amounts of IRS-1; 3) analyzed for potential binding proteins using Surface Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI_TOF_MS); 4) ran through SDS_PAGE and staining (Colloidal Blue and Ruby Red) to identify potential binding proteins visually; and 5) planned to be analyzed by Liquid Chromatography-Mass Spectrometry Mass Spectrometry (LCMS_MS) to identify any positive reads from staining procedures.

Unfortunately, no positive reads could be identified by Colloidal Blue or Ruby Red staining. There were far too many nonspecific proteins (present in all the samples) obstructing view of any of the hypothesized IRS-reset-specific phosphatases. Since no bands could be identified, none could be cut out, digested, and identified by LCMS_MS. To solve this problem, the next set of samples will be centrifuged after cell lysing and only the clear lysate will be analyzed, better focusing on which proteins to analyze. Still, Colloidal Blue staining produced a visible band at ~24 kDa: likely GRB-2, an insulin-stimulated protein. Also, preliminary SELDI_TOF_MS found what appear to be two reset-specific proteins at ~14 kDa. These successes will prove to be the first step toward the eventual identification of an IRS protein-protein reset complex.

MYOSTATIN EFFECT ON C2C12 MYOBLAST DIFFERENTIATION

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Objective. Myostatin (Mst) is a novel muscle growth factor that belongs to the transforming growth factor (TGF)-beta superfamily. Mst or growth differentiation factor-8 negatively regulates muscle mass. Increased expression of myostatin has been associated with loss of muscle in conditions that cause muscle wasting such as HIV-infection, aging, cancer, immobilization and spaceflight. However, the primary role of Mst in regulating muscle mass remains poorly understood. The purpose of this study was to identify the endogenously expressed Mst effect on cell differentiation, especially on myoblast formation.

Methods. To overexpress the Mst protein in C2C12 myoblast, we used a cDNA construct in which a wild type muscle creatine kinase (MCK) promoter sequence was placed upstream of either mouse myostatin cDNA (MCK-Mst construct) or the green fluorescent protein (GFP) reporter gene (MCK-GFP construct). The two constructs were co-transfected into myoblast cells at the ration of 4:1. The transfection efficiency was followed by determining the number of green fluorescent cells.

Conclusion. The data demonstrated that increased expression of myostatin in the muscle cells or tissues is associated with reduced capability of cell differentiation, which could result in muscle wasting in vivo.

EFFECTS OF DIABETIC NEUROPATHY ON SYNAPTIC SUMMATION

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Diabetes is associated with neurological disorders such as impaired attention, executive function, visuo-spatial memory, and verbal memory in human patients. In rodents, streptozotocin-induced diabetes has been shown to alter the subunit composition of sodium channels in neurons. Furthermore, induced diabetic conditions contribute to changes in current amplitude and activation kinetics of sodium channels. We hypothesized that such changes could contribute to modifications in neuronal information processing. It has been previously demonstrated that voltage-gated ion channels, including sodium channels, in neuronal dendrites directly influence the integration of multiple synaptic inputs. This is based on the fact that these ion channels can amplify excitatory postsynaptic potentials and allow action potentials to propagate back into the dendrite. The temporal patterns of synaptic input carry information crucial to neuronal information processing. We studied the effects of varied sodium channel conductance densities and activation kinetics on temporal summation of several realistic neuron models. The altered temporal summation patterns showed that increased conductance densities cause an increase in maximum summation at synchronous inputs. Furthermore, a negative shift in activation kinetics generates large-amplitude, cell-wide, all-or-none responses while the control generates a small-amplitude, local, graded response. These altered temporal summation patterns could explain some clinical observations of neuropathy in diabetic patients.

DEPRESSION, HEALTH BELIEFS, AND LOCUS OF CONTROL: RELATIONSHIPS TO GLYCEMIC CONTROL IN HISPANICS AND AFRICAN AMERICANS WITH TYPE 2 DIABETES

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In the United States today, 6.2% of the population (17 million people) have diabetes. Depression has been found to be 2 to 3 times higher in patients with diabetes compared to patients without diabetes. Studies have also found that patients with both depression and diabetes have an increased number of complications and poor glycemic control. Moreover, this concern is especially critical in the underprivileged and under-served minority communities that continue to struggle with an epidemic of undiagnosed and untreated mental disorders. Thus, it is imperative to find ways to reach critical populations to make successful interventions.

The correlation between glycemic control in Latino and African American patients, their health beliefs, and mental health were evaluated in this study. This cross-sectional study recruited subjects from a randomized list of patients with diabetes in the King/Drew Medical Center (KDMC). The subjects responded to Beck's Depression Inventory, the Health Belief Scale, and Multi-Dimensional Health Locus of Control. These instruments are used to diagnose depression, explain and predict a patient's preventive health behaviors, and to measure the degree to which a patient believes his/her health is controlled by internal or external factors.

This study is a continuation of a three-year study. There are many social cultural factors that may contribute to the poor health management of diabetes. Hence, this study is designed to assess the health management of the under-served minority patients with diabetes. This year, an additional 25 surveys have been collected; our total number of respondents is now 100. As in previous years, 51% of the respondents suffer from depression.

EXPRESSION OF VANILLOID RECEPTORS (TRPV1) IN VISCERAL DORSAL ROOT GANGLIA (DRG) NEURONS FROM RATS

Student Researcher: Jonathan Black, King/Drew Magnet High School of Medicine and Science
Mentor: Victor Chaban, PhD, Charles R. Drew University of Medicine and Science, Los Angeles, California

Defining the sites and mechanisms through which sex steroids modulate nociception is an important step in understanding and treating pain and designing appropriate therapies. The objective of our study was to investigate the expression of vanilloid receptors (TRPV1), which play a significant role in the nociception at the lumbar-sacral (L_1-S_3) levels of the DRG. Retrogradely labeled DRG cells were examined to determine if these neurons receive input from both colon and the uterus. Fluorogold tracer was micro-injected into the colon/rectum and tetramethylrhodamine bilaterally into the uterine horns of Long-Evans rats. Ganglia were harvested, cryoprotected and cut in 20 μm slices for fluorescent microscopy to identify labeled cells. Up to 5% neurons were colon-specific or uterus-specific and 10%–15% of labeled DRG neurons were innervate both viscera in the L_1, L_2, L_6 and S_1–S_3 levels. DRGs sections were immunostained with rabbit-generated primary antibodies against TRPV1 (Neuromics, Northfield, Minn) for 48 hours, washed in 0.01 M PBS with 0.1% Triton X, and then incubated for 80 min in donkey anti-rabbit IgG conjugated to FITC (Molecular Probes, Eugene, OR) followed by washes in PBS. Sections were mounted on slides and coverslipped with Vectashield (Vector Laboratories, Burlingame, Calif). Our data suggest that viscerally labeled DRG neurons (both colon-specific and uterus-specific) express TRPV1 receptors and suggest a novel form of visceral sensory integration, which may explain the mechanism of many functional pain syndromes.

THE ROLE OF CAVEOLIN-1 IN THE ALLERGY-INDUCED CHANGES IN EXTRACELLULAR MATRIX

Student Researcher: MaatiK Ka'awa, Kaunakakai, Hawaii
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Asthma is a chronic inflammatory disease of the airways characterized notably by reticular basement membrane thickening and is associated in some patients' airway remodeling. Airway inflammation in allergen-challenged mice is associated with increased cytokines expression, activation of the transforming growth factor beta (TGFβ) signaling, and increased expression of extracellular matrix proteins. TGFβ has anti-inflammatory effects and stimulates collagen deposition. Its expression is significantly increased in patients with chronic asthma. By interacting with TGFβ receptors, caveolin-1 (cav1) inhibits TGFβ signaling. We hypothesize that TGFβ activity and matrix protein synthesis is increased in cav1null mice.

To induce airway inflammation, 8-week old C57BL/6J and cav1-deficient mice were sensitized by intraperitoneal injection with either phosphate buffer saline (PBS) or ovalbumin (OVA) at day 0 and 14. The mice were then challenged by inhalation with the same allergen at days 24, 26 and 28 and sacrificed at day 29. Lung tissue was imbedded in paraffin and 5 microns sections were cut. To investigate the activation of the TGFβ signaling pathway, TGFβI receptor immunostaining and a Masson's trichrome stainings for collagen deposition were performed. Tissue sections were stained with alcian blue for mucus secretion. TGFβ receptors were detected in all mice. Increased collagen deposition and mucus secretion were shown in C57BL/6J challenged mice. PBS-challenged cav1deficient mice already show evidence of mucus secretion and collagen deposition, which were not enhanced after challenge. By studying TGFβ signaling pathways, we will provide better information to treat and prevent airway remodeling, a late-phase complication of asthma.

INVESTIGATION OF TEA-DERIVED NUTRIENTS ON CARDIOVASCULAR DISEASE

Student Researcher: Tiffany Lastimosa, James Campbell High School
Mentors: Joan Kuh, PhD, and Andre Theriault, PhD, University of Hawaii, Honolulu, Hawaii

Faulty overproduction of lipids and apolipoprotein B (apoB) by the human liver is associated with increased levels of low density lipoprotein (LDL), also known as bad cholesterol. Understanding how nutritional factors down-regulate LDL-apoB levels can lead to new methods of lowering the risk of developing cardiovascular disease (CVD) associated with type 2 diabetes. My project focused on different tea-derived nutrients called catechins from green tea and theaflavin from black tea. The hypothesis was that various tea-derived nutrients would inhibit LDL-apoB production using human liver cells (HepG2) grown in culture. This was tested using an immunoassay called ELISA, which detects the protein component of LDL called apolipoprotein B (apoB) secreted into the tissue culture media. Our results indicated that cells treated with theaflavin from black tea slightly inhibited apoB secretion. The green tea catechins (ie, epigallocatechin gallate [EGCG], epicatechin [EC], epicatechin catechin [ECC], epicatechin gallate [ECG]) inhibited the secretion of apoB, with EGCG showing the most potent effect of all tea nutrients. Therefore, our *in vitro* study has concluded that green tea catechins, particularly EGCG, to be more potent than black tea in lowering the risk of developing CVD associated with type 2 diabetes. This finding could lead to further studies in animals and humans in the form of dietary intervention trials.

COMPARATIVE GENOMICS OF GROUP A *STREPTOCOCCUS* ISOLATED FROM HAWAIIAN PATIENT

Student Researcher: Leon Hou, Iolani School
Mentors: Guliz Erdem, MD, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii; Maqsudul Alam, PhD, Department of Microbiology, University of Hawaii, Honolulu, Hawaii

Infections caused by group A *Streptococci* (GAS) are very common and range from the relatively benign, such as pharyngitis, to the potentially lethal, such as necrotizing fasciitis. One complication resulting from GAS infections is acute rheumatic fever (ARF). ARF is significantly more prevalent in Hawaii than in the rest of the United States, particularly among people of Native Hawaiian and Polynesian descent. GAS isolated from ARF patients in Hawaii seem to frequently feature *emm* types that are rare in the continental United States. Little research has been conducted on rare *emm* types in the past. We have initiated two genome sequencing projects, one of a GAS strain isolated from a Polynesian patient with ARF and one of a reference strain of *emm* type 122. We created the random shotgun libraries for these two genome sequencing projects and was involved in high throughput sequencing and partial assembly for the reference strain. In our analysis, we focused on the genomic analysis of the *emm* region and on virulence-related ORFs. These virulence factors could potentially serve as indicators of infections and/or rheumatogenicity caused by the strain. The comparative genomics analysis results will provide a better understanding of GAS genetics and biology and will have further relevance to studies of host-pathogen interactions. Results are pending.

NICOTINE INDUCES PROLIFERATION IN HUMAN MESANGIAL CELLS: A NOVEL LINK BETWEEN CIGARETTE SMOKING AND RENAL DISEASE

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In the United States, the major cause of preventable mortality is cigarette smoking. Cigarette smoking is well known to be a major risk factor for coronary artery disease, peripheral vascular disease, and lung disease, including emphysema and lung cancer. Recent clinical studies have also suggested that, in addition, cigarette smoking is a risk factor in the progression of renal disease; however, the mechanisms involved are not known. We designed experiments to determine whether nicotine, an important component of tobacco, has growth-promoting effects on human mesangial cells and whether these effects are mediated by cytokines, which are known to mediate cell proliferation such as platelet-derived growth factor (PDGF). Mesangial cell proliferation in response to nicotine (10^{-7} to 10^{-12}) was assessed by ^{3}H-thymidine incorporation after 24 hours stimulation. To determine the role of PDGF on the growth of nicotine, we measured PDGF concentrations by ELISA in the cell supernatant of cells exposed to nicotine and adjusted for cell protein content. Nicotine, in a dose dependent manner, increased mesangial cell proliferation as assessed by ^{3}H- thymidine incorporation. However, these effects were not accompanied by significant increase in PDGF, suggesting that this cytokine is not a significant mediator of these effects. These studies demonstrate that nicotine promotes mesangial cell proliferation and therefore unveil previously unknown mechanisms that may participate in the progression of chronic kidney disease in smokers.

CHANGES IN GFAP IMMUNOREACTIVITY FOLLOWING IMMUNOLOGIC LESIONS IN THE RAT BRAIN

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Mentor: Sonsoles de Lacalle, MD, PhD, Charles R. Drew University of Medicine and Science, Los Angeles, California

Alzheimer's disease (AD) is a complex, irreversible and progressive brain disorder that occurs gradually and results in memory loss, and behavior and personality changes. One region of the brain that is always damaged is the basal forebrain cholinergic system. Many investigators have shown that cholinergic neurons have a role to play in cognitive processes. For this reason, it is believed that cholinergic cell loss may be involved in the memory changes experienced by AD patients. Previous studies from our laboratory have suggested that estrogen may have a neuroprotective effect, particularly with respect to maintaining dendritic arborizations. Other studies have shown that estrogen increases the number of dendritic spines. Along with estrogen as a possible source of therapeutic intervention for AD, we are also interested in assessing the role of glial cells, specifically in the involvement of astrocytes in this neurodegenerative process. We hypothesized that there could be a possibility for astrocytes to be beneficial to the cholinergic neurons. We used immunocytochemistry against glial fibrillary acidic protein (GFAP), a specific marker for astrocytes, to study the role of these supporting cells. If, in fact astrocytes are involved in the neuroprotective process, then we expect to see an increase in GFAP immunoreactivity following a lesion in the rat brain. Results are pending.

DIABETES-INDUCED DYSREGULATION OF ENDOTHELIAL PROTEINS IN A TYPE I MURINE MODEL

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Mentors: J Gregory Maresh, PhD, RalphV Shohet, MD, University of Hawaii John A. Burns Medical School, Honolulu, Hawaii

Objective. Diabetes-associated effects on protein regulation within the aortic endothelium were assessed in a murine model of type I diabetes. Our main experimental aim was to assess alterations of endothelial protein levels occurring within a mouse model type I diabetes. Prior studies within our laboratory involving microarray and real-time PCR analysis of FACS-sorted endothelium indicate a >20 fold increase in mRNA transcript levels of GlyCAM-1 in mice rendered diabetic for 21 days.

Methods. Aortic thin sections of diabetic and control aortae were examined using immunohistochemistry to assess endothelial localization of eNOS and GlyCAM-1, using commercially available anti-eNOS and MECA-79 antibodies. SDS-PAGE of aortic and heart proteins, followed by anti-eNOS Western blotting, was included in our study as a biochemical measure of endothelial dysfunction and SDS-PAGE of serum, followed by MECA-79 western blotting, was performed to measure Glycam-1.

Results. By immunohistochemistry, no apparent differences were found in either GlyCAM-1 or eNOS in response to diabetes, although immunolocalization was visible within the endothelium. Preliminary western blot results indicated readily detectable eNOS dimer and monomer within protein extracts of the heart and aorta. Quantification of differences in dimer:monomer ratio in response to diabetes of await further study. GlyCAM-1 is readily detectable by western blotting within serum of control mice, but measurement of differences due to diabetes await further studies.

THE CLEAR PLAQUE GENES OF THE BACTERIAL VIRUS, EPSILON-34 (E34)

Student Researcher: NataliedB Rivera, Thomas Armstrong Toro High School
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In this project, we worked with the bacteriophage ε^{34} that infects the bacteria *Salmonella*. The normal phage produces cloudy plaques when grown in bacterial cultures. In our laboratory, we have made a clear plaque mutant phage, ε^{34} c99. Our laboratory has determined the DNA sequence of the ε^{34} c99 DNA and our hypothesis is that mutations in the C1, C2 and/or C3 genes are responsible for the clear plaque that is produced by the mutant phage. To identify mutations in ε^{34} c99 (mutant phage), we sequenced the mutated DNA of the C1, C2 and C3 regulation genes. We submitted the obtained sequences to the NCBI database for confirmation of identities. The amino acid sequence analyses showed that the ε^{34} c99 CI protein is 70% identical to the CI protein of the Stx2 bacteriophage; the CII protein is 30% homologous to the CII protein of the bacteriophage HK97; and the CIII protein is a 94% identical to the *Salmonella typhimurium* ST64T. In the next step of this project, we will work to find out if the C genes are mutated in the ε^{34} c99 phage DNA and if they are responsible for the clear plaque formation.

IDENTIFICATION OF GENES THAT REGULATE LIPID METABOLISM AND LONGEVITY EXTENSION BY DIETARY RESTRICTION

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More than 70 years of research has shown that dietary restriction in mice lengthens lifespan and ameliorates diseases such as cancer and diabetes. The biological mechanism through which diet exerts its effects, however, has remained elusive. Previous investigation of genes that control both natural defense mechanisms and aging in model systems has led to the idea that the gene Sir2 (Sirt1 in mammals) may mediate the lifespan extension brought about by dietary restriction. Sir2 has been shown to be involved in lipid storage and metabolism; this observation led us to hypothesize that the beneficial effects of dietary restriction are mediated through processes involving lipid metabolism. We tested our hypothesis by examining how mutations in individual genes influence fat storage and mobilization in the fruit fly, Drosophila melanogaster. We first examined the role of Sir2 in this process by comparing the triglyceride content of mutant Sir2 and control flies that were starved and subsequently re-fed. We also executed a genome-wide screen designed to identify novel genes that influence obesity, aging, and enhanced health. We concluded that the gene, Sir2, does not significantly influence lipid mobilization, but may affect the rate at which the animals regain lipid content. Our data also suggest sex differences in the ability of flies to mobilize and store fat. A screen of 400 different mutations has led to the identification of two novel genes involved in lipid metabolism. A better understanding of these mutant genes will yield new information about diseases and treatments related to human aging.

SYNTAXIN 6 INHIBITS EPITHELIAL CA2+ CHANNELS TRPV5 AND TRPV6

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Epithelial Ca2+ channels TRPV5 and TRPV6 are involved in active Ca^{2+} absorption in the kidney and intestine, respectively. Ca^{2+} enters across the apical membrane of Ca^{2+} transporting epithelial cells through these channels, and then exits through Ca^{2+} pumps and/or sodium-Ca^{2+} exchangers in the basolateral membrane. Thus, TRPV5 and TRPV6 are gatekeepers of the transcellular Ca^{2+} transport pathway. To transport Ca^{2+} across the apical membrane, these channels must be delivered to the plasma membrane to be functional after biosynthesis. Since TRPV5 and TRPV6 are constitutively active, their protein levels at the plasma membrane determine their Ca^{2+} transport activity. In this study, we investigated to what extent syntaxin affects the activity and membrane expression of TRPV5 and TRPV6 in the *trans*-Golgi network. Syntaxins are small membrane proteins that are involved in membrane fusion of vesicles. Using Ca^{2+} uptake influx and voltage-clamp techniques, we found that syntaxin 6 strongly inhibited TRPV5 and TRPV6 mediated Ca^{2+} transport and current. With western blot analysis, we found that syntaxin 6 prevented the complex glycosylation of TRPV5 and TRPV6. This is likely due to the blockade of insertion of the channel proteins into the plasma membrane. Our conclusion is that syntaxin 6 increases the retention of immature TRPV5 and TRPV6 channel proteins in the Golgi complex so that the levels of mature channel proteins in the plasma membrane are reduced.

THE EFFECTS OF ENVIRONMENTAL ENRICHMENT AND FLAVONOIDS ON HYPERTENSION IN SHR RATS

Student Researcher: RaphaelA Malbrue, Catholic High School
Mentor: Egeenee Daniels, DVM, University of North Texas Health and Science Center- Laboratory Animal Department, Baton Rouge, Louisiana; CT Rabi, DVM, MS, Plank Road Animal Hospital, Baton Rouge, Louisiana.

Hypertension, also known as the silent killer, occurs when a person's blood pressure is consistently ≥140/90 mm Hg. Hypertension usually has no signs or symptoms and causes the heart to work too hard. Several factors have been linked to high blood pressure, eg, heredity, ethnicity, obesity. One in every three United States adults has high blood pressure and does not even know it. An inactive lifestyle without regular exercise increases one's chances of hypertension. Hypertension can lead to several other problems such as strokes, impaired vision, kidney damage, congestive heart failure, and heart attacks. In our research, we separated SHR rats, genetically selected, to become hypertensive into Group A and Group B. Our goal was to study and record how stress and flavonoids (agents found in dark chocolate) affect high blood pressure. Group A was termed the "happy rats" and Group B were the "stressed" rats. Results are pending.

UMBILICAL CORD BLOOD (HUCB) CELLS: THERAPEUTIC PROMISE IN ISCHEMIC VICTIMS

Student Researcher: Justus Roberts II, Paul R. Wharton High School
Mentors: Alison Willing, Paul Sanberg, Jennifer Newcomb, College of Medicine, University of South Florida, Tampa, Florida

The purpose of this study was to determine whether human umbilical cord blood (hUCB) cells alter indicators of inflammation outside of the brain, including white blood cell type and apoptosis (cell death) expressed in the peripheral blood, spleen and thymus after a stroke. The overall goal of this research was to develop a treatment for strokes. The treatment is the delivery of hUCB, which may decrease the inflammatory components of the disease. The hypothesis was that stroke will change the peripheral immune system and the hUCB cells will have a positive change, returning the immune response to normal. The peripheral blood was separated using a Ficoll gradient separating the red blood cells, white blood cells and plasma, the white blood cells were extracted and cultured. The spleens and thymuses, from rats who were normal, rats with stroke, and rats with stroke and ab injection of hUCB cells, were homogenized. Enzyme-linked immuno-adsorbant assay (ELISA) was done on the cultures and homogenates. Immunohistochemistry experiments were performed on spleens and thymuses, then observed under a fluorescent-microscope. The inflammation and apoptosis decreased and the sizes of the organs and T-cell (white blood cell) count increased, back to their original states, after the introduction of hUCB cells in animals with stroke. These results are consistent with a decrease in inflammation, which could decrease the secondary inflammation-induced injury in the brain. Therefore, hUCB cells could potentially be a treatment for stroke victims, other defects, infections, and diseases.

CLEAVAGE OF THE WEST NILE VIRUS NONSTRUCTURAL 4B PROTEIN PLAYS A ROLE IN MEMBRANOUS WEB FORMATION IN HUMAN KIDNEY EPITHELIAL CELLS

Student Researcher: Angelica Rockquemore, NIH/NIDDK/Drew Summer High School Program 2006
Mentors: VivekR Nerurkar, PhD, PakieliH Kaufusi, PhD, Retrovirology Research Laboratory, Department of Tropical Medicine, Medical Microbiology, and Pharmacology, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii

Background. West Nile virus (WNV) is a positive single-stranded RNA virus of approximately 11 kb encoding three structural and seven non-structural (NS) proteins. Nonstructural 4B protein (NS4B), the least characterized protein of WNV, resides in the endoplasmic reticulum (ER) and initiates formation of the membranous web (MBW) structures where the virus presumably replicates. We hypothesized that NS4B protein blocks ER stress upon binding to activating transcription factor 6 (ATF6), a regulator for ER stress response genes. Moreover, ATF6 activates cleavage within NS4B protein, but not at the signalase site at 2K/NS4B, to initiate MBW formation.

Materials and Methods. The C- or N-terminal of WNV NY99 strain NS4B carrying the 44- or 17- amino acids to C-terminus of NS4A (2K) was fused to green fluorescence protein (GFP) in the expression vector pcDNA3.1/CT-GFP-TOPO, and expressed in HEK 293 cells. Cell viability was assessed using trypan blue dye exclusion and proliferation assays. To visualize the expression of NS4B-GFP, we employed fluorescence and confocal microscopy as well as immunoblotting. The ER stress was measured by quantitating the amount of transcripts of the ATF6 activated GRP78 and Trebb genes using quantitative real time reverse-transcriptase PCR (qRT-PCR).

Results and Conclusions. Expression of NS4B-GFP in HEK293 cells exhibited a distinct reticular pattern with small isolated MBW structures in the cytoplasm that were positive for ER markers. The signal peptide (2K) is not required for MBW formation. Cell viability assay data indicated decrease in cells expressing NS4B protein. By using immunofluorescence, triton X-114 phase separation, and immunoblotting, the NS4B-GFP was localized to the ER membrane where C-4B and C-sig4B were cleaved producing approximately 54- and 38-kDa peptides. Concomitant appearance of MBW and the 38-kDa peptide were revealed with fluorescence microscopy and immunoblotting, respectively. The 38-kDa peptide is hypothesized to be associated with the MBW. qRT-PCR data indicated that NS4B did not induce ER stress indicating that NS4B might block cell stress to initiate MBW formation. Our preliminary data coupled to the data available in the literature strongly suggest that NS4B is the best target for development of WNV antiviral therapy.

THE ROLE OF BMP IN THE MOUSE INCISORS' STEM CELLS

Student Researcher: Shareez Sher Ali, Clements High School
Mentors: Margarita Bonilla-Castillo, James F. Martin, MD, PhD, Texas A&M University Institute of Bioscience and Technology, Houston, Texas

Adult mouse incisors have the unique characteristic of growing continuously as the result of new enamel matrix deposition from the ameloblast. A population of multipotent cells has been recognized and located in the epithelial cervical loop (CL) of the apical end of the incisor. These multipotent progenitors proliferate and differentiate into ameloblast, stratum intermedium, stellate reticulum, and outer epithelium cells. So far, some molecules have been identified in order to understand what molecular signaling controls this multipotent cell population in the CL. FGF-10 deficiency has been shown to cause defects in stem cell compartmentalization and induced apoptosis in CL cells[1]. BMP signaling has been recognized to be involved in the regulation of other stem cell environments. For example, BMP signaling is found to control the niche size in the hematopoietic system and, in Drosophila, it interrupts the differentiation in germ line stem cells. We hypothesized that BMP signaling through the BMPR1A is important for maintaining the mouse incisors stem cells by controlling their self-renewal capacity. In order to explore the role of BMP signaling in stem cell self-renewal, a BMPR1A conditional mutant embryo was generated using the cre/lox-p system. We performed histology analysis using wild-type and BMPR1A conditional mutant incisors to compare the proliferation and apoptotic status of the cells at the CL. Results are pending.

OVEREXPRESSION OF HEME OXYGENASE-1 PROTECTS AGAINST SIMVASTATIN INDUCED MUSCLE TOXICITY

Student Researcher: VanishaR Yarbrough, Alabama School of Fine Arts
Mentors: Marcienne M Wright, Anupam Agarwal, MD, Department of Medicine, Nephrology Research and Training Center, University of Alabama at Birmingham, Birmingham, Alabama

Background. Statins refer to a family of drugs that aid in the reduction of the amount of LDL cholesterol within the body. Although statins protect against cardiovascular disease, these drugs cause damage to muscle cells causing inflammation, pain, and acute kidney failure due to the release of muscle proteins into the blood stream. One form of statin, simvastatin, induces the production of heme oxygenase-1 (HO-1), an enzyme responsible for the breakdown of heme into iron, carbon monoxide, and biliverdin. In addition to acting as a catalyst, HO-1 has been found to protect cells from damage and inflammation.

Objective. We studied whether overexpression of HO-1 would decrease muscle cell damage by simvastatin.

Methods. Mouse C_2C_{12} muscle cells were treated with simvastatin at various concentrations after the cells over expressed HO-1. HO-1 overexpression was achieved in three ways: adeno-associated viral (AAV) infection, hemin induction, and HO-1 expressing plasmid DNA transfection. After treatment with control (vehicle) and simvastatin, the live/dead cell viability assay determined the number of living cells within the treated and control cells. An unpaired t-test was used to determine if there was a significant difference among cells with and without HO-1. The HO-1 expressed through AAV infection protected the cells at a concentration of 100 μM of simvastatin. HO-1 induced by hemin protected the cells consistently at a concentration of 25 μM and 50 μM of simvastatin.

Results. The results from the induction of HO-1 induction through the creation of stable cell lines by DNA transfection are pending. The initial results with AAV or hemin induction of HO-1 indicated that overexpression of HO-1 in muscle cells is protective against simvastatin-induced muscle toxicity.

ESTROGEN EXPRESSION IN THE MEDIAL AMYGDALA

Student Researcher: Yesenia Chavez, King/Drew Magnet High School of Medicine and Science
Mentor: SamanthaS Gizerian, PhD, Charles R. Drew University of Medicine and Science, Los Angeles, California

Background. In the brain, both subtypes of the estrogen receptor, ER-a and ER-b have been known to be expressed abundantly in the medial amygdala. The active form of estrogen, 17-b estradiol (E2), partly regulates ER expression by binding to the ER, a subtype. ER expression is known to be increased in response to traumatic injury and it is an early step in the injury response. It is unknown whether the same pattern occurs after an immunotoxic insult as it has been shown to do after trauma.

Objective. The objective of this study was to determine whether ER expression was altered after an immunotoxic or exogenous estrogen treatment.

Methods. Our study was conducted on previously prepared rat tissue samples. These rats had been castrated (GDX), ovariectomized (OVX), and others were left intact (NGDX and NOVX) and implanted with a subcutaneous pellet containing either E2 or placebo (P). Immunostaining procedures using antibodies directed against ER-a and ER-b were utilized. The resultant patterns of staining were examined microscopically and the average change in ER-b expression was calculated.

Results. Our data showed that the change in ER-b expression increased in animals that were treated with E2. These results show that ER expression is increased after an immunotoxic insult.