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Book of abstracts

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Breakout Session IIB: Immunology & Tumor Suppression / 7

RNA binding proteins regulate innate immune response to influenza virus

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The 1918-1919 H1N1 influenza pandemic resulted in millions of human deaths worldwide, and severely impacted isolated communities in Alaska and the Pacific. Today, pathogenic avian influenza A viruses remain capable of crossing from avian reservoirs into humans, causing severe pulmonary disease characterized by pneumonia, high levels of inflammatory cytokines and chemokines, and immunopathology. Avian influenza viruses must typically acquire molecular adaptations in their heterotrimeric RNA-dependent RNA polymerase (PB1, PB2 and PA) and nucleoprotein (NP) to efficiently synthesize RNA in human cells. Using functional genomics (RNA interference), biological network analysis, and phylogenetics, we have explored interactions of H1N1, H5N1 and H7N9 viral polymerases with a network of human RNA binding proteins. Cellular RNA binding proteins, including heteronuclear ribonucleoporoteins (hnRNP) and DExD/H-box RNA helicases, regulates influenza virus RNA syntheses in human cells, suggesting a role for RNA binding proteins in the adaptation of RNA viruses to mammalian hosts. Intriguingly, RNA binding proteins, such as PKR, hnRNPA1, and NF90, regulate RNA virus replication and also potentially induction of type I interferon and cellular RNA stress responses, possibly by binding immunostimulatory viral RNA motifs. Targeting these virus-host interactions may offer a new line of therapeutic intervention against severe RNA virus infections in humans.

Poster Session - Board 056 / 8

Impact of the K24N mutation on the transactivation domain of p53 and its binding to MDM2

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Background:
The level of the p53 transcription factor is negatively regulated by the E3 ubiguitin ligase murine double minute clone 2 (MDM2). The interaction between p53 and MDM2 is essential for the maintenance of genomic integrity for most eukaryotes. Previous structural studies revealed that MDM2 binds to p53 transactivation domain (p53TAD) from residues 17 to 29. The K24N mutation of p53TAD changes a lysine at position 24 to an asparagine. This mutation occurs naturally in the bovine family and is also found in a rare form of human gestational cancer called choriocarcinoma.
Objective:
In this study we have investigated how the K24N mutation affects the affinity, structure, and dynamics of p53TAD binding to MDM2.
Methods and Results:
Nuclear magnetic resonance studies of p53TAD show the K24N mutant is more flexible and has less transient helical secondary structure than the wildtype. Isothermal titration calorimetry measurements demonstrate that these changes in structure and dynamics do not significantly change the binding affinity for p53TAD-MDM2. Finally, free energy perturbation and standard molecular dynamics simulations suggest the negligible affinity change is due to a compensating interaction energy between the K24N mutant and MDM2 when it is bound.
Conclusion:
Overall, the data suggests that the K24N-MDM2 complex is able to at least partly compensate for an increase in the conformational entropy in unbound K24N with an increase in the bound state electrostatic interaction energy.

Breakout Session IB: Development & Reproductive Biology / 10

The milder DNA damage and abnormal chromatin packaging phenotype in Sly deficient mice is partially due to the residual retention of SLY protein.

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Background: The Y-specific (non-PAR) region of the mouse Y chromosome long arm (NPYq) encodes multiple copies of at least 4 distinct genes expressed in spermatids: Ssty, Sly, Asty, Orly. Mice with NPYq deletions have sperm defects and are either sub- or infertile, with the severity of the phenotype increasing proportionally to the deletion size. To assess which of the NPYq genes is responsible we produced mice, in which the function of NPYq encoded Sly has been disrupted by a transgenically delivered siRNA. The characterization of these shSLY mice revealed presence of sperm head anomalies, infertility, chromatin packaging defects and increased sperm DNA damage similar to that noted in NPYq deletion mutants but less severe.

Objective: Our goal was to determine if the milder 'sperm phenotype' of shSLY mice was due to insufficient SLY knockdown.

Methods: To address this we developed a new anti-SLY antibody, as the previous analyses of SLY protein expression were hampered because previously used serum recognized only one of the two existing SLY isoforms. Peptide specific for both SLY isoforms (SLY1&2), was used to immunize mice using a standard approach. The serum was tested in ELISA, dot-blot, and western blot with protein lysates from HEK293 cells transfected with Sly1 and Sly2 ORFs fused to FLAG tags. The serum was SLY specific, recognized both existing SLY isoforms, and was transformed into a monoclonal anti-SLY antibody. This antibody was used in western blots with protein lysates prepared from testes obtained from mice with NPYq deletions and from shSLY mice.

Results: Mice with a partial NPYq deletion and slight phenotype had most of SLY1 (98%) and SLY2 (70%) retained. Mice with extensive NPYq deletions and severe phenotype had no identifiable SLY1 and SLY2. Two shSLY lines were examined. Line sh344, with no phenotype, retained only 5% of SLY1 but almost the entire SLY2 (96%). Line sh367, with a moderate phenotype, retained 4% of SLY1 and 6% of SLY2.

Conclusion: Together, these data provide evidence that a milder sperm phenotype of sh367 mice results, at least partially, from the retention of residual SLY1/2. Our results also support the important role of SLY2, which becomes a target for our future investigations. Expression data do not preclude the possibility that lack of another NPYq encoded gene contributes to the severe phenotype of mice with extensive NPYq deletions.

[Supported by HD072380 and NIH P20RR024206-01 (project 2) grants to M.A.W.]

Poster Session - Board 069 / 11

Assessing barriers and enhancers to increasing physical activity during the school day in children on an American Indian Reservation: A qualitative research study

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Obesity and the development of diabetes has become a major heath concern for American Indian (AI) tribal communities in the United States. The purpose of this study was to determine community-identified strategies that enhance or limit physical activity (PA) during the school day in 4th, 5th, and 6th grade children attending an elementary school on an AI reservation in the Northwestern United States. Six community focus group discussions (FGD) were conducted and led by trained researchers from the University of Montana in May and June 2012. The FGD's consisted of students in the 4th, 5th and 6th grade and adult community members. Each FGD contained 7-11 participants, lasted approximately 60 minutes and recorded. The audio-recordings of the FGD's were transcribed and analyzed utilizing Grounded Theory. All the strategies that the participants identified were included in the analysis and used to generate themes, sub-themes, and sub-theme elements. Two major themes for barriers to PA emerged from the analysis: 1) the intense school-day format prevents the opportunity for exercise, and, 2) the school lacks resources that might increase physical activity. Three major themes for PA strategies emerged from the analysis: 1) structured competitive activities, 2) structured non-competitive activities, and, 3) increasing school and community-wide capacity that is supportive and conducive to increasing daily PA. The findings from this formative study were used to design a school-based PA intervention for the elementary school children.

Poster Session - Board 013 / 12

Wound repair in preimplantation embryo after trophectoderm injury

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Background: Preimplantation development extends from fertilization to implantation into the wall of the uterus. It culminates in the generation of the blastocyst, which consists of tissues called the trophectoderm (TE) and pluripotent inner cell mass whose function are placentation and body formation, respectively. The blastocyst has a central cavity that expands to increase TE interaction with the uterine wall during implantation. Human assisted reproductive technologies (ART) are procedures that aid infertile couples to conceive. It includes in vitro fertilization, and recently, it is being conducted along with preimplantation genetic diagnosis (PGD). PGD involves TE biopsy, that is, the removal of several TE cells from a blastocyst, which are analyzed for genetic anomalies. Thus, TE biopsy makes it possible to select for genetically normal embryos for uterine transfer into patients. In spite of the potential benefits of TE biopsy, it involves inflicting injury to the embryo, which causes blastocyst cavity collapse and reduction in cell number. Some successful births have been reported after embryos have undergone TE biopsy. Nevertheless, since there are no guidelines or regulations of TE biopsy, it is unclear what extent of injury the embryo can withstand and whether the cavity collapse affects pregnancy outcomes.

Objective: The long-term goal is to assess the impact of TE biopsy that involves significant injury to the embryo.

Methods: The mouse embryo was used as a model to assess the impact of cavity collapse on blastocysts. Cavity collapse was induced by two different methods: mechanical injury versus cytochalasin D (CD) treatment. Recovery process of collapsed blastocysts was monitored using time-lapse video microscopy. Cell death due to mechanical injury was assessed by live fluorescence staining using propidium iodide (PI).

Results: When blastocysts were punctured, the TE retracted towards the ICM side, which brought the edges of the wound in proximity to each other. Most of the collapsed blastocysts began to recover their cavity within 1 hour after puncture, and they became similar in size to control blastocysts within 5-6 hours later. PI-positive dead cells emerged around the site of injury, but they were excluded from the TE epithelium during recovery of blastocysts. CD-treated collapsed embryos also recovered efficiently upon withdrawal of CD from the culture medium.

Conclusion: Blastocysts were competent to undergo wound healing in spite of TE injury, as evidenced by recovery of the cavity. This suggests that repair mechanisms are activated when the TE epithelium is damaged. Our data have wider implication for improving efficiencies in human ART that impact the quality of embryo development in vitro. Future studies are warranted, such as transfer of injured blastocysts into surrogate mothers, to investigate the impact of TE biopsy on the efficiency of implantation and fetal development.

Breakout Session IIIC: Health Disparities / 13

Community-based ecotoxicology with indigenous peoples

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Background: This talk explores two case studies of community-based participatory research with indigenous peoples of Alaska and Australia examining the ecological and health consequences of contaminant exposure.
Objective: The Alaskan case study, with the Yupik people of St. Lawrence Island and Norton Sound villages, examines endocrine disruption due to PCBs, PFCs, PBDEs and chlorinated pesticides and uses stickleback and Alaska blackfish as model organisms. The Australian case study examines the ecotoxicology of manganese on Groote Eylandt, a sensitive ecosystem, and uses both fish and marsupial models.
Methods: We employ analytical chemistry to determine tissue levels of contaminants, protein biomarkers to test for disruption of sex steroids, and histology to test for disruption of the thyroid axis.
Results: Chemical analyses of fish from St. Lawrence Island indicate elevated levels of PCBs at Northeast Cape, a formerly used defense site (FUDS) that has been subject to substantial cleanup efforts. Male fish collected at FUDS in villages along Norton Sound express elevated levels of vitellogenin, indicating estrogenic activity by xenobiotics.
Conclusion: Both the Alaskan and Australian case studies involve participatory research by indigenous peoples and capacity building through on-site training. Levels of specific PCB congeners at Northeast Cape on St. Lawrence Island indicate contamination from the FUDS rather than elevated PCB levels due to global distillation. Analyses are now being expanded to study gene expression and genomic evolution of fish from contaminated vs. control sites. The Australian project is in its infancy, but the participatory research format from Alaska is being applied to study manganese contamination on Groote Eylandt with the Anindilyakwa Rangers.

Breakout Session IIIA: Infectious Diseases - Viruses / 14

HCMV Replication in ATM Deficient Cell Lines is Dependent Upon Virus Strain and Host Cellular Environment

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Human cytomegalovirus (HCMV) is the leading viral cause of birth defects. Following HCMV infection the cellular DNA damage response (DDR) is upregulated, but DNA damage recognition and repair does not proceed to completion. Ataxia telangiectasia mutated (ATM) is a signal transduction molecule critical to double-strand break (DSB) signaling. Our previous studies determined that HCMV Towne replicated as efficiently in an ATM- cell line (GM02530) as in wildtype (wt) fibroblasts (HFF). Subsequent studies using HCMV AD169 showed diminished functional virion production in a different ATM- cell line (GM05823), suggesting that ATM is required for efficient virion production. To clarify these disparate findings we studied the progression of the Towne and AD169 lifecycle in HFFs and 3 ATM- cell lines (GM02530, GM05823, GM03395). We found that functional virion production varied drastically between cells with similar ATM- phenotypes and as a function of virus strain. Both virus strains produce virions efficiently in HFF cells while neither virus strain replicates well in GM03395, as would be expected if ATM were required for virion production. However, infection of GM02530 ATM- cells produces wt levels of functional virions with both virus strains. Perhaps most interestingly, infection of GM05823 yields both a productive (Towne) and a non-productive (AD169) infection, indicating that additional factors contribute to the efficiency of HCMV virion production in these ATM- cells. We identified similar defects in virion trafficking to the nucleus and replication center formation during AD169 infection of both GM05823 and GM03395 cell lines. In contrast, virion trafficking out of the nucleus is deficient in Towne-infected GM03395 cells. Efficient virion production was not determined by the presence of functional ATM, but rather as a function of the interactions of the individual virus strain with the unique cellular environments of the ATM- lines. Our study indicates that further characterization of the interactions of viral strains with ATM- lines may be a powerful tool to advance our understanding of HCMV's interactions with its cellular environment.

Poster Session - Board 021 / 15

Cathepsin K Deficiency Attenuates Starvation-Induced Cardiac Autophagy and Apoptosis

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Background: Cathepsin K, a lysosomal cysteine protease, is up-regulated in the heart in response to pressure-overload and knockout of cathepsin K protects against cardiac dysfunction associated with obesity and hypertension although the molecular mechanism involved in the process is unclear.
Objective: Here we evaluated the effect of genetic ablation of cathepsin K on starvation-induced cardiac dysfunction.
Methods: Wild-type and Cathepsin K knockout (Ctsk-/-) mice were subjected to 65h starvation or free access to food. Cardiac function was assessed. Western blot analysis and co-immunoprecipatation assay were used to detect the expression of autophagy and apoptosis markers. TUNEL staining was used to indicate cardiomyocyte apoptosis. H9c2 cells were transfected with adenovirus-LC3 and challenged by glucose deprivation in the presence or absence of cathepsin K siRNA to investigate autopahgy. Furthermore, apoptosis markers were detected in glucose deprivation challenged H9c2 cells with or without cathepsin K silencing.
Results: Wild-type mice following 65h starvation displayed impaired cardiac function as evidenced by decreased fractional shortening and enlarged left ventricle end diastolic dimension which were reconciled by deletion of cathepsin K. In addition, starvation resulted in single cardiomyocyte contractile dysfunction and impaired intracellular Ca^{2+} handling in wild-type mice both of which were alleviated by cathepsin K knockout. At the molecular level elevated cardiac autophagy was observed following starvation, which was shown to be detrimental for cardiac dysfunction under starvation as 3-methyladenine, an autophagy inhibitor was able to rescue starvation-induced cardiac dysfunction. Although autophagy was also activated in the starved cathepsin K knock out mice, the autophagic flux was disrupted as evidenced by the dissociation of beclin 1/Bcl 2 complex, and the accumulation of LC3BII and p62 protein. Starvation resulted in increased cardiomyocyte apoptosis, assessed as positive TUNEL staining and changes in the protein levels of Bax, Bcl2 and PARP, which were attenuated in the cathepsin K knockout mice. In cultured H9c2 cells, starvation autophagy as assessed by the accumulation of adenovirus-LC3B punta, which was accentuated on silencing cathepsin K. Similarly, increased apoptosis was observed in the starved H9c2 cells, which was rescued by inhibiting cathepsin K.
Conclusion: Thus, the beneficial effects of cathepsin K knockout in the heart may be attributed to the disruption of autophagic flux and inhibition of apoptosis.

Breakout Session IIA: Cardiovascular / 16

Cardiomyocyte-Specific Knockout of PTEN Induces Hypertrophic Cardiomyopathy: the Role of Autophagy

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Background— The role of phosphatase and tensin homolog deleted from chromosome 10 (PTEN) in maintaining cardiac homeostasis is still controversial. While some studies suggest that muscle-specific loss of PTEN triggers cardiac hypertrophy and cardiomyocyte contractile dysfunction, others indicate that muscle-specific PTEN deficiency protects against pressure overload-induced heart failure. Recent studies have suggested that autophagy, an evolutionarily conserved pathway for protein degradation, is regulated by PTEN via mammalian target of rapamycin (mTOR).
Objective— This study was designed to evaluate the role of cardiomyocyte-specific PTEN in the maintenance of cardiac homeostasis and underlying mechanisms, with a focus on autophagy.
Methods and Results—We generated mice with cardiomyocyte-specific knockout of PTEN, α-MHC Cre-PTEN(flox/flox) (CM PTENKO). These adult PTEN-/- mice recapitulated evidence of hypertrophic cardiomyopathy (HCM), including geometric, functional, histological and molecular changes. We found especially that cardiomyocyte-specific PTEN knockout increased cardiac mTOR but suppressed autophagy activity.
Conclusion—Our results demonstrate an essential role of cardiomyocyte PTEN in maintaining the cardiac homeostasis under physiological conditions. Cardiomyocyte-specific loss of PTEN leads to the development of hypertrophic cardiomyopathy, which may be caused by mTOR hyperactivation and autophagy suppression. Additionally, our data suggest that mTOR inhibitors may be considered as a potential therapeutic treatment of patients with PTEN haploinsufficiency suffering from hypertrophic cardiomyopathy.
Supported by NIH P20 RR016474

Poster Session - Board 052 / 17

Detection and Characterization of Human Polyomavirus JC in the People of Hawaii

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Background: Progressive multifocal leukoencephalopathy (PML) is a fatal demyelinating disease caused by the human polyomavirus JC (JCV), which predominantly affects immunocompromised individuals. While archetype JCV is prevalent in the healthy human population, only rearranged type of JCV causes PML. Worldwide approximately 80% of healthy individuals are seropositive for JCV, and about 30% of these individuals excrete archetype JCV in their urine. The objective of this study is to collect baseline JCV prevalence data among healthy individuals from Hawaii and later correlate with JCV prevalence among immunocompromised individuals. Methods: Urine samples were collected from healthy volunteers after obtaining informed consent and the age, gender, and ethnicity of the study participants were noted. Cells were then isolated from urine by centrifugation and DNA was extracted followed by amplification of JCV non-coding control region (NCCR) using PCR. The amplified products were then visualized on an agarose gel. Results and Conclusions: Ten urine samples from healthy individuals have been collected, five males and five females, whose ages ranged from 19 to 55. Urine processing and PCR-data analysis is ongoing.

Poster Session - Board 041 / 18

Human apolipoprotein A-I is associated with dengue virus and enhances virus infection through SR-BI

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Diseases caused by dengue virus (DV) infection vary in severity, with symptoms ranging from mild fever to life threatening dengue hemorrhage fever (DHF) and dengue shock syndrome (DSS). Clinical studies have shown that significant decrease in the level of lipoproteins is correlated with severe illness in DHF/DSS patients. Available evidence also indicates that lipoproteins including high-density lipoprotein (HDL) and low-density lipoprotein (LDL) are able to facilitate cell entry of HCV or other flaviviruses via corresponding lipoprotein receptors. In this study, we found that pre-incubation of DV with human serum leads to an enhanced DV infectivity in various types of cells. Such enhancement could be due to interactions between serum components and DV particles. Through co-immunoprecipitation, we revealed that apolipoprotein A-I (ApoA-I), the major protein component in HDL, is associated with DV particles and is able to promote DV infection. Based on that observation, we further found that siRNA knockdown of the scavenger receptor class B type I (SR-BI), the cell receptor of ApoA-I, abolished the activity of ApoA-I in enhancement of DV infection. This suggests that ApoA-I could bridges DV particles and cell receptor SR-BI and facilitates entry of DV into cells. FACS analysis of cell surface dengue antigen after virus absorption further confirmed that ApoA-I enhances DV infection via promoting initial attachment of virus to cells. These findings illustrate a novel entry route of DV into cells, which may provide insights into the functional importance of lipoproteins in dengue pathogenesis.

Poster Session - Board 017 / 19

Genetic risk factors for GDM and preeclampsia in women from Hawaii

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Background: Preeclampsia and GDM increase the risk of developing cardiovascular disease and type 2 diabetes (T2DM), respectively, in both the acute and remote postpartum periods. Among Asian and Pacific Islander populations gestational diabetes mellitus (GDM) and preeclampsia are highest in women of Filipino descent, followed by Chinese, Japanese and Native Hawaiian/Pacific Islanders. Objective: We choose to investigate single nucleotide polymorphisms (SNP) previously associated with T2DM and cardiovascular disease in our local pregnant population. In this proposal we aim to identify polymorphisms in several genes either associated with GDM or with preeclampsia in Hawaii minority populations. Methods: OpenArray technology (Applied Biosystems) will be used to screen maternal DNA from RMATRIX Biorepository for 32 single nucleotide polymorphisms (SNPs). Chi-squared test statistics and ORs were calculated to determine SNP associations between recessive or dominant genetic models and GDM and/or preeclampsia. Results: In the Filipina population, six SNPs demonstrated a dominant inheritance pattern and three SNPs a recessive pattern with GDM. One SNP demonstrated a dominant pattern with preeclampsia. In the Pacific Islander population, two SNPs demonstrated a dominant pattern and one SNP a recessive inheritance pattern with GDM. Two SNPs demonstrated a dominant pattern and two a recessive inheritance pattern with preeclampsia. Conclusion: In the Filipina and Pacific Islander populations genetic polymorphisms previously associated with T2DM and cardiovascular disease are also associated with GDM and preeclampsia. These SNPs may serve as a link or as predictors, between the development of GDM and preeclampsia and the later development of T2DM and cardiovascular disease.

Poster Session - Board 039 / 20

Contribution of the Intestinal Microbiome to the Development of Hirschsprung's-Associated Enterocolitis

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1. Background: Hirschsprung's disease (HD) is a congenital malformation of the gastrointestinal tract characterized by the absence of the distal enteric nervous system, Hirschsprung's-associated enterocolitis (HAEC) is one of the most serious complications of HD.

2. Objective: Our objective is to characterize the structure of the microbiome in HD patients by performing a longitudinal study that encompasses episodes of HAEC and remission.

3. Methods: Our experimental design is to enroll five HD patients at the time of diagnosis with HAEC. Fecal samples will be collected prior to antibiotic treatment, after completion of antibiotic treatment, three months after treatment and six months after treatment. The samples will be frozen and pulverized. Extraction of genomic DNA will be performed using a bead-beating technique, which will be followed by phenol-chloroform extraction. The microbiome will be characterized by 16S rRNA gene pyrosequencing. The data obtained will be used to taxonomically classify and compare community structure between samples.

4. Results: We expect that the structure of the microbiome within an individual patient in active HAEC will differ from that seen in remission. We do not expect that different patients will show exactly the same disease-related structures, although we may observe similar trends between patients.

5. Conclusion: If the results support the hypothesis, the data will help to form strategies to positively impact the morbidity and mortality of HAEC. This research might also help to understand microbial contributions to other serious intestinal inflammatory diseases.

Breakout Session IIC: Infectious Diseases - Bacteria & Fungi / 21

Structural analysis and innate immune responses of the atypical O-antigens in Burkholderia pseudomallei, the causative agent of melioidosis.

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Background
Burkholderia pseudomallei is the causative agent of melioidosis that causes major public health problems in Southeast Asia and northern Australia. Currently, there is no available effective vaccine. Each B. pseudomallei strain expresses one of four distinct O-antigen moieties of lipopolysaccharides (LPS) known as types A, B, B2, and rough. Strains with O-antigen types B, B2, and rough are identified as atypical strains which are not common in Southeast Asia, but found significantly in northern Australia. O-antigen types B and B2 are serologically related, but not cross-seroreactive with the typical type A. Rough type does not produce ladder patterns of the O-antigen on SDS-PAGE and is not seroreactive. Chemical structures of the atypical O-antigens are unknown.

Objective
We investigated the chemical structures for both atypical O-antigen types B and B2, and their innate immune responses to compare with the phenotypes expressed by the typical type A.

Methods
Various analytical techniques including GC/MS and NMR were used to determine the O-antigen structures. We studied the innate immune responses of the LPS samples purified from types A, B, and B2 B. pseudomallei strains and its near-neighbors in RAW246.7 mouse macrophages.

Results
Both LPS types B and B2 triggered greater nitric oxide and cytokine production than those from type A. With a combination of glycosyl composition using GC/MS and linkage analyses with 1D and 2D 1H and 13C NMR, we determined the structures of the B. pseudomallei type B O-antigen as: [β-Xylp-(1→4)-3OAc-β-Rhap-(1→4)-α-Rhap-(1→3)-α-Galp-(1→]n and the type B2 O-antigen as: [3)-2OAc-β-Rhap-(1→4)-α-Rhap-(1→3)-α-Galp-(1→]n.

Conclusions
All three O-antigen types, A, B, and B2 of B. pseudomallei are structurally different. The atypical types B and B2 are more sensitive to host innate immunity than those of the typical type A. This would suggest that the typical strains may be more successful in escaping from the host defenses than the atypical strains. We believe that these O-antigenic differences may cause distinct immunogenic - pathogenic life styles between the typical and atypical B. pseudomallei strains.

Breakout Session IB: Development & Reproductive Biology / 22

Possible Role for Origin Recognition Complex in Maintaining Origin Sites in the Zygote

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INTRODUCTION: In order to efficiently replicate the genome, potential replication origins must be licensed with a complex of proteins that begins with the origin recognition complex proteins (ORC2-5) and ends with the helicases (MCM2-7). We have recently shown in the mouse zygote, licensing appears to be completed in the ovulated MII maternal DNA prior to fertilization, while paternal licensing occurs de novo and completed shortly after pronuclear formation. It has previously been shown that DNA synthesis is inhibited in cyclohexamide (CHX) treated parthenotes, as well as with the injection of antisense RNA to Orc6 mRNA into GV oocytes. OBJECTIVE: In this work, we tested the function of ORC2 translation in the zygote. METHODS and RESULTS: MII oocytes were incubated in CHX [40μg/mL] for 30 min, which partially activates oocytes, and tested for the presence of ORC2 and MCM7 by immunocytochemistry (ICC) at 2 hrs, 4 hrs, and 8 hrs after 10 mM SrCl2 activation. We observed that ORC2 was present between the anaphase II maternal DNA on a spindle-like structure, while MCM7 associated more directly to the separating chromatin, as observed in untreated zygotes. During G1, 4 hrs after activation, the maternal pronucleus contained both ORC2 and MCM7, as we also previously reported. However, unlike normal developing zygotes, by 8 hrs after activation during the time DNA synthesis is normally occurring, the maternal pronucleus becomes devoid of ORC2 but still has a strong ICC MCM7 signal despite not having undergone replication. We repeated these experiments with zygotes created by ICSI and found similar results with one important exception. The paternal pronucleus retained some ORC2 even at 8 hrs after fertilization. ICSI generated zygotes were treated with 100 mM NaCl at 4 hrs and both the ORC2 and MCM7 were extracted from the pronuclei, suggesting they were not bound to DNA in G1 despite being present in the pronuclei. CONCLUSIONS: The results, taken together with our previous work, which proposed replication origins were licensed by 2 hrs after fertilization, suggest that continuous ORC2 translation during zygotic G1 is required to maintain the licensed state of replication origins. To the best of our knowledge, such a surveillance function for ORC2 has not been previously suggested. This work was supported by NIH grant HD060722.

Poster Session - Board 059 / 23

Protein tyrosine phosphatase 1B and insulin resistance: Role of endoplasmic reticulum stress/reactive oxygen species/nuclear factor kappa B axis

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Background and Aim: Obesity-induced endoplasmic reticulum (ER) stress has been proposed as an important pathway in the development of insulin resistance. Protein-tyrosine phosphatase 1B (PTP1B) is a negative regulator of insulin signaling and is tethered to the ER-membrane. The aim of the study was to determine the mechanisms involved in the crosstalk between ER-stress and PTP1B. Methods and Results: PTP1B whole body knockout and C57BL/6J mice were subjected to a high-fat or normal chow-diet for 20 weeks. High-fat diet feeding resulted in increased body weight gain, adiposity, systemic glucose intolerance, and hepatic steatosis, which were attenuated by PTP1B deletion. High fat diet- fed PTP1B knockout mice also exhibited improved glucose uptake measured using [3H]-2-deoxy-glucose incorporation assay and Akt phosphorylation in the skeletal muscle tissue, compared to their wild-type control mice which received similar diet. High fat diet-induced upregulation of glucose-regulated protein-78, phosphorylation of eukaryotic initiation factor 2α and c-Jun NH2-terminal kinase-2 were significantly attenuated in the PTP1B knockout mice. Mice lacking PTP1B showed decreased expression of the autophagy related protein p62 and the unfolded protein response adaptor protein NCK1 (non-catalytic region of tyrosine kinase). Treatment of C2C12 myotubes with the ER-stressor tunicamycin resulted in the accumulation of reactive oxygen species (ROS), leading to the activation of protein expression of PTP1B. Furthermore, tunicamycin-induced ROS production activated nuclear translocation of NFκB p65 and was required for ER stress-mediated expression of PTP1B. Conclusion: Our data suggest that PTP1B is induced by ER stress via the activation of the ROS-NFκB axis which is required for the unfolded protein response in mediating insulin resistance in the skeletal muscle under obese condition.

Breakout Session IC: Infectious Diseases - Parasites & Vectors / 24

Increasing Ambient Temperature and Susceptibility of the Mosquito Aedes aegypti to the Insecticide Permethrin: What's Global Warming Got to Do with It?

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Background: Previous studies show that temperature directly affects the toxicity of insecticides to insects. Organophosphates exhibit a positive correlation between ambient temperature and mortality for many insect species. Carbamates exhibit a slightly negative correlation between ambient temperature and mortality. In contrast, pyrethroids are known to exhibit a distinctly negative correlation between increasing ambient temperature and mortality for many insect species. However, this relationship has not been systematically studied for adult mosquitoes.

Objective: Therefore, we are examining the influence of temperature on the susceptibility of adult Aedes aegypti when exposed to the Type I pyrethroid, permethrin.

Methods: We are characterizing the median lethal concentration, LC50, for adult Ae. aegypti when exposed to eight concentrations of permethrin for 24 hours in bottle assays. In addition, we are determining the LC50 and dose-response curves for adult Ae. aegypti when exposed to the same concentrations at four temperatures (16, 23, 30, 34 oC) for 24 hours.

Results: Preliminary results show a negative correlation between temperature and mortality with temperatures between 16 oC and 30 oC and a positive correlation with temperatures from 30 oC to 34 oC 24 hours after exposure to permethrin.

Conclusion: If mosquito populations are expanding in space and time because of increased temperatures due to climate warming, and at the same time they cannot be managed as effectively with pyrethroids, then this may pose considerable risk to public health.

Breakout Session IB: Development & Reproductive Biology / 25

Ureteropelvic junction obstructions in mice with conditional inactivation of exocyst Sec10 in kidney and upper urinary tract epithelium.

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Background:
Congenital obstructive nephropathy, the most common cause of pediatric chronic kidney disease and end stage renal disease (ESRD), is caused by obstruction of the urinary tract during fetal development. The most common cause of congenital obstructive nephropathy is ureteropelvic junction obstruction (UPJ obstruction), when the stenosis is localized to the upper urinary tract where the renal pelvis transitions into the ureter. Despite the high prevalence and medical burden of UPJ obstructions, we have a poor understanding of its molecular and genetic basis, with a scarcity of non-surgical genetic animal models.

Objective:
To identify how molecular and cellular defects in urinary tract epithelial cells contribute UPJ obstructions.

Methods:
In previous studies using cell culture models, we have shown the eight-protein exocyst trafficking complex to be critical in maintaining aspects of polarized epithelial cells. To further in vivo studies of polarized exocytosis in renal development and disease, we have generated a novel transgenic mouse to conditionally knockout Sec10, a central component of the exocyst. This is the first conditional mouse strain for any exocyst subunit, and should be valuable in studying the exocyst's role in various tissues and diseases.

Results:
We crossed this floxed-Sec10 strain with the Ksp-Cre mouse strain to induce a knockout in Sec10 specifically in ureteric bud-derived epithelial cells during embryonic development. Surprisingly, 90% of the Sec10FL/FL;Ksp-Cre conditional knockout mice died quickly after birth, displaying severe bilateral hydronephrosis due to congenital obstructions of the upper urinary tract. From histological analysis, these blockages were due to an overgrowth of the surrounding smooth muscle cells at the UPJ, with complete disappearance of the ureter lumen. From immunohistochemistry of the mutant urothelial cells in the upper ureter, we show an absence of uroplakin-3, one of the proteins critical to maintaining the fluid-impermeable barrier of the urothelial cells that line the ureters, bladder, and renal pelvis.

Conclusions:
The exocyst plays a crucial role in urothelial cell differentiation and/or barrier function during urinary development. This novel transgenic mouse model of UPJ obstruction may be valuable for further studies of the causes and consequences of human congenital obstructive nephropathy.

Poster Session - Board 001 / 26

Coordination and Step Rate During Forelimb & Hindlimb Stepping in the Neonatal Rat: Effect of Quipazine Dose

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BACKGROUND: Quipazine (a serotonergic agonist) has been shown to induce locomotor behavior, including alternated stepping, in perinatal and spinally injured rodents. OBJECTIVE: This study aimed to identify the most effective dose of quipazine for evoking alternated stepping behavior in postnatal day 1 (P1) rats. METHODS: Pups were tested in a 35-min session. Following a 5-min baseline, pups were treated with quipazine (1.0, 3.0, or 10.0 mg/kg) or saline (vehicle control), administered intraperitoneally in a 50-microliter injection. Behavior was recorded for the next 30 minutes and scored during playback. RESULTS: All doses of quipazine evoked alternated fore- and hindlimb stepping, however the 3.0 and 10.0 mg/kg doses of quipazine evoked the highest frequencies and proportions of steps. To further examine stepping at these doses, interlimb phase and bilateral step rate were calculated. There was no difference in interlimb phase between the 3.0 and 10.0 mg/kg quipazine doses. For bilateral step rate, there also was no difference between the 3.0 and 10.0 mg/kg doses. CONCLUSION: Overall, these findings suggest that the 3.0 and 10.0 mg/kg quipazine doses may be used to effectively promote alternated limb coordination in future experiments with newborn rats.

Poster Session - Board 057 / 27

Cloning the carboxy-domain of the PDI9 cDNA from Arabidopsis thaliana and over-expression of the fusion protein in E. coli

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Background: Protein disulfide isomerases (PDIs) catalyze the formation, breakage, and rearrangement of disulfide bonds to properly fold nascent polypeptides within the endoplasmic reticulum (ER). PDI9 is one of 12 PDI genes found in the Arabidopsis genome.

Objective: The objective was to express and purify the PDI9 c terminal fusion protein containing His·Tag for generation of an antibody and identification of its subcellular location and function within the cell.

Methods: A ~530bp 3' region encoding the carboxy-end of the PDI9 protein was amplified by PCR using PDI9 cDNA template. The 3' DNA fragment was then cloned into a pET-15b vector. Recombinant plasmids were transformed into E. coli (DH5α) and sequenced. One construct with the correct PDI9 sequence, in-frame with the vector-specific His·Tag N terminus, was transformed into E. coli (BL21) cells and used for over-expression of the fusion protein.

Two sets of experiments were performed to determine the optimal temperature and time, and final IPTG concentration for induction. Four 5ml cultures grown to OD600=0.6 were induced with a final concentration of 0.5mM IPTG for 1, 3, 6, and 18 hours at room temperature, 30°C, and 37°C. An SDS-PAGE performed indicated strong induction at room temperature for 18 hours. Four 5ml cultures were grown again to OD600=0.6 and were induced with a final concentration of 0.25, 0.5, 0.75, and 1mM IPTG at room temperature for 18 hours.

Results: Optimal conditions for over-expression of the fusion protein were found to be 1mM IPTG final concentration and an 18-hour induction at room temperature. A Western blot analysis showed that the primary antibody against His·Tag reacts strongly to the over-expressed 21.1kDa protein band in the blot.

Based on the results, a 100ml batch of the transformed E. coli cells was grown to OD600=0.6 and induced with a final concentration of 1mM IPTG at room temperature for 18 hours. Cells were lysed and lysate obtained was then combined with Ni-NTA His Bind resin and passed through chromatography columns to obtain elute fractions of the fusion protein. Resulting SDS-PAGE indicated a residual protein of approximately 100 kDa remaining along with the fusion protein. To remove the residual protein, elute fractions from the chromatography columns were passed through Amicon Ultra 50K or 30K centrifugal filters. A clean protein was visible following SDS-PAGE and Western blot analysis.

Conclusion: The objective of expressing a clean PDI9 c terminal fusion protein was accomplished. The next step is to grow a large 500ml batch for induction and cleave off the His·Tag sequence before it can be used to make antisera in rabbits.

Poster Session - Board 003 / 28

Characterization of intracellular signaling in an in vitro presynaptic model expressing nicotinic receptors on prolonged exposure to beta amyloid

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Background: Amyloid beta (Aβ) is the main constituent of neuritic plaques found in the brains of patients with Alzheimer's disease (AD). Under physiological conditions, picomolar levels of soluble Aβ have been proposed to play a neuromodulatory role primarily via activation of synaptic nicotinic acetylcholine receptors (nAChRs). At higher concentrations (nM-μM), as found in AD patients, prolonged exposure to Aβ may cause synaptic disruption and, eventually, neuronal injury. Among various downstream targets of Aβ are the molecules involved in synaptic function, memory formation and cognition, such as MAP kinases ERK and JNK, MKPs, CaMKII, Akt, Fyn and Tau.
Objectivez: To assess the activation and interaction of these various signaling molecules upon prolonged exposure to Aβ in cells expressing nAChRs in order to characterize the steps leading to neurotoxicity. One particular focus was on the involvement of the activated signaling molecules in oxidative stress.
Method: The rodent neuroblastoma cell line NG108-15 was used as neuronal model system, which, upon differentiation, forms axonal presynaptic-like varicosities. Mouse sequences for the α4- and β2-nAChR subunits housed in expression vectors were transiently transfected into differentiated NG108-15 cells for 48 hrs. The cultures were then exposed to Aβ1-42 for different time-points (up to 3 days). Extracted proteins were subjected to gel electrophoresis (4-20% polyacrylamide), transferred to blots. The blots were probed using phospho-specific and total protein antibodies for the various proteins of interest. Image-iT Live detection was used to determine the levels of reactive oxygen species (ROS) and nuclear integrity.
Results: Our studies showed substantial and time-dependent activation of ERK in response to nM Aβ exposure at a very early time point (30min). CaMKII activation was also an early event, but it was not sustained, unlike ERK activation which continued to increase over several days as did Akt activation. pERK upregulation was followed by JNK activation as well as an increased expression of PHF-tau and Fyn. In parallel, long-term exposure to nM Aβ1-42 induced oxidative stress, measured as increased levels of ROS in the cells, but was only significant in neurons transfected with α4β2 nAChRs as compared to mock-transfected cells. The impact of prolonged Aβ on the levels of ROS was attenuated by the MEK-selective inhibitor U0126. In addition, the MEK inhibitor attenuated Aβ-induced nuclear fragmentation, which was found to follow the changes in ROS levels in response to Aβ.
Conclusion: These results demonstrate that the presence of nAChRs sensitizes neurons to the neurotoxic action of Aβ through the activation of discrete intracellular signaling pathways. Furthermore, our results indicate that different pathways may converge at a very early stage of Alzheimer's disease.

Breakout Session IC: Infectious Diseases - Parasites & Vectors / 29

Development of pattern recognition receptor ligands as malaria vaccine adjuvants

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Background: A critical obstacle to malaria vaccine development is the lack of appropriate adjuvants to elicit potent and protective immunity. Recent insights into the role of pattern recognition receptors (PRRs) in dendritic cell (DC) activation and identification of follicular helper T (Tfh) cells, as specialized providers of B cell help, enable a novel approach to adjuvant selection. The overall goal of our study is to identify new molecular adjuvants and adjuvant combinations that may be used to promote Tfh cell differentiation.
Objective: The objective of our study is to identify new molecular adjuvants and adjuvant combinations that may be used to activate DCs and drive them to stimulate Tfh cells to provide help for the antibody response to malaria antigens. Our general hypothesis is that specific combinations of PRR agonists will be effective as molecular adjuvants by inducing dendritic cells to activate Tfh cells to promote a potent and biologically-active antibody response to P. falciparum blood stage antigens
Methods: We evaluated PRR ligands that activate through distinct intracellular signaling pathways: a Toll-like receptor (TLR) agonist signaling through the MyD88-dependent pathway (R848), TLR agonists signaling through the TRIF-dependent pathway (p(I:C) and GLA); an agonist of Nod1 (C12-iE-DAP) that signals through RIP2; and an agonist for Mincle (synthetic trehalose-dibehenate, TDB) that signals through FcR Src-family kinase. Mouse bone marrow-derived DCs were stimulated with various PRR ligands, individually or in combination, for 3-8 h (RNA expression studies) or 12-24 h (protein studies). RNA transcription was measured by real-time PCR and protein expression was measured by flow cytometry and multiplex cytokine assays.
Results: R848 was a strong inducer of DC activation in vitro, particularly when combined with p(I:C), inducing significantly higher cytokine RNA (IL-12, TNF-alpha) and protein (IL-12, TNF-alpha, IL-6, IFN-gamma), producing higher DC expression of CD86, and activating both pDCs and cDCs. IL-6, a potent Tfh polarizing cytokine, was maximal in DC cultures stimulated with R848-containing combinations. PRR stimulation also enhanced ICOS ligand expression by DCs, with R848 being most effective stimulator in inducing ICOS ligand positive DCs. However, GLA was distinctive in its ability to induce a high ICOS ligand density DC subset that was not observed with other PRR agonists.
Conclusion: Several PRRs and PRR combinations were effective stimulators of DC activation and differentiation. However, subtle differences were observed in surface expression of DC differentiation markers. Ongoing studies will evaluate the ability of selected PRR combinations to polarize naïve CD4+ T cells to differentiate into Tfh cells in vitro and in vivo and provide help for P. falciparum blood-stage vaccines.

Poster Session - Board 004 / 30

Synaptic regulation by a N-terminal fragment of beta amyloid

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Background: Soluble β-amyloid (Aβ), a key protein in Alzheimer's disease, has been shown to regulate presynaptic Ca^{2+} and synaptic plasticity. In particular, picomolar β-amyloid was found to have an agonist-like action on presynaptic nicotinic receptors (nAChR) and to augment long-term potentiation (LTP). The precursor protein of Aβ (APP) is differentially cleaved in vivo by the actions of α- and/or β- and/or γ-secretases as well as resident carboxypeptidases, notably producing multiple hydrophilic N-terminal fragments present in the brains and CSF of both healthy adults and Alzheimer's patients. The physiological significance and function of these hydrophilic N-terminus fragments have not been examined. Our objective was therefore to discover the functional activity and conformational state, if any, of the N-terminal Aβ fragments.

Methods: Live Ca^{2+} confocal imaging studies were performed whilst perfusing N-terminal Aβ fragments: in vitro using the rodent neuroblastoma cell line NG108-15 which were differentiated and then transfected with α7-nAChR; ex vivo using rodent synaptosomes. Electrophysiological examination of acute exposure to N-terminal Aβ fragments on long-term potentiation (LTP) was performed using mouse hippocampal slices. Contextual fear conditioning was performed after bilateral injection into dorsal hippocampi of mice of the 1-15 N-terminal Aβ fragment, saline as a control or the selective α7-nAChR blocker MLA just prior to the administration of the fragment. Tris-Tricine native gel electrophoresis together with and without prior centrifuge filtration using 3kDa filters of purified peptides was performed to assess oligomeric status. The secondary structures of the N-terminal fragments were assessed using CD spectral analysis.

Results: Ca^{2+} imaging demonstrated that the functional domain in Aβ exists within the N-terminal region. A N-terminal fragment of Aβ encompassing residues 1-15 proved to be over twice as effective and significantly more potent than full-length Aβ in its agonist-like action on nAChRs. This activation was shown to require the critical Tyr-188 in the agonist binding domain of α7-nAChR. Picomolar but not nanomolar concentrations of the 1-15 N-terminal Aβ fragment augmented contextual fear conditioning and this augmentation was specific to the action of α7-nAChR, as it was blocked by the prior administration of the selective α7-nAChR blocker MLA. In mouse hippocampal slices LTP was enhanced by femtomolar but not higher concentrations of the 1-15 N-terminal Aβ fragment. Despite running anomalously on SDS gels, the N-terminal fragments were shown to be stable monomers. No stable secondary structures were found to be present in the 1-15 N-terminal Aβ fragment upon examination by CD spectral analysis.

Conclusion: These findings suggest that the N-terminal β-amyloid fragment may serve as a potent and effective neuromodulator.

Poster Session - Board 082 / 31

The New Mexico (NM) INBRE Sequencing and Bioinformatics Core

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Background: The National Center for Genome Resources (NCGR) is a non-profit research institute whose mission is to improve human health and nutrition through bioinformatics and DNA sequencing. NCGR has been at the forefront of bioinformatics since 1994 when it developed the first relational genome sequence database (GSDB), and thereafter with a series of innovative software tools and practices integrating and extracting value from –omics data. By establishing a Next Generation Sequencing (NGS) Center in late 2007, and being the first Illumina sequencing Certified Service Provider (CS-Pro®) in North America in 2008, NCGR and the NM INBRE Sequencing and Bioinformatics Core (SBC) are transforming the research of the INBRE network by providing de novo sequencing, resequencing and functional genomics applications of enormous translational impact. These include the molecular understanding of single-gene and complex disorders, cancer biology, traits of agronomic importance, environmental genomics and personalized medicine. This insight is achieved by combining NGS with the center's sophisticated bioinformatics tools, software development, robust IT infrastructure and creative techniques for data mining and knowledge discovery.

Objective: To provide the INBRE network with turn-key access to discovery-enabling sequencing and bioinformatics solutions, educational symposia, mentorship/internship programs and collaborative research support.

Methods: NCGR is a recognized Illumina and Agilent CS-Pro certified lab with sequencing instrumentation comprised of two HiSeq2000's, a GAIIx, and a Pacific Biosciences RS. The lab uses the PerkinElmer (Caliper) LCGX high-throughput nucleic acid assessment instrument in conjunction with the Sciclone NGS liquid handling robot to QC samples and prepare libraries in a high-throughput manner. Sequencing services include whole genome and transcriptome shotgun, ChIP, small RNA, mate-pair, ultra-low sample input (1ng) DNA, and targeted exome. Bioinformatics includes de novo assembly (transcriptome and genome), read count-based expression, variant detection, differential expression analysis and custom bioinformatics for challenging analysis problems. A competitive pilot study RFP mechanism is used to establish collaborations in the INBRE network to solve pressing research questions in various organisms and clinical areas.

Results: Since 2008, over 45 INBRE collaborations have been created enabling 15 grant awards and 12 peer reviewed publications in the areas of basic science and environmental research, assessment and modeling of human disease (in traditional and emerging model organisms), de novo genome assembly and comparative genomics of agents of infectious disease, and biological studies in human disease.

Conclusion: The NM-INBRE SBC increases the research capacity of INBRE investigators and their competitiveness in attaining research grants and manuscript publications by providing cutting-edge discovery techniques, fruitful collaborations, a yearly educational New Mexico BioInformatics, Science, and Technology (NMBIST) symposium, and bioinformatics-based educational initiatives for elementary through graduate school students.

Grant Support: NIGMS (8P20GM103451-12).

Breakout Session IIIA: Infectious Diseases - Viruses / 34

West Nile virus-induced cell adhesion molecules on the brain microvascular endothelial cells are critical for the transmigration of peripheral leukocytes into the brain

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Backgrpund: West Nile virus (WNV), a positive strand RNA virus, is the major cause of mosquito borne encephalitis in the United States. WNV neuropathogenesis is dependent on the ability of the virus to enter the central nervous system (CNS). Increased CNS infiltration of leukocytes, specifically CD8+ T cells are critical for clearing virus infection from the brain, although infiltrating inflammatory monocytes are also proposed to contribute to the neuropathology. WNV uses several strategies to cross the blood-brain barrier (BBB) including directly infecting the brain microvascular endothelial cells or via infected leukocytes that enter the CNS, however the associated mechanisms are unclear. Cell adhesion molecules (CAMs) on the vascular endothelial cells are implicated in leukocyte trafficking into the brain in several neuroinflammatory diseases. Our previous work has demonstrated that WNV infection induced expression of CAMs such as ICAM-1, VCAM and E-selectin in human brain microvascular endothelial cells (HBMVE) and that matrix metalloproteinase's (MMPs) secreted by infected human astrocytes mediate degradation of BBB tight junction proteins (TJP). Recently, we have also showed that WNV infection in vivo causes degradation of TJPs, which correlates with peak virus titers, MMPs and infiltrated leukocytes in the brain of mice.

Methods: To extend our previous studies, the objective of this study was to employ a well-established in vitro BBB model using HBMVE cells to characterize the role of WNV-induced CAMs in the infiltration of the leukocytes into the CNS. Adhesion and transmigration of human PBMC derived monocytes and monocytes-depleted leukocytes across the in vitro BBB model was analyzed in the presence or absence of neutralizing antibodies against these CAMs.

Results: Adhesion of mock-infected leukocytes increased dramatically to the WNV-infected HBMVE cells, which reduced significantly in the presence of neutralizing antibody cocktail (VCAM, ICAM-1 and E-selectin). Further, disruption of the integrity of WNV-infected in vitro BBB model using HBMVE cells following transmigration of leukocytes was observed to decrease dramatically in the presence of neutralizing antibody cocktail. On the other hand, incubation of infected leukocytes with mock-infected in vitro BBB model did not result in any significant change in the integrity of in vitro BBB model as compared to un-infected leukocytes. Comparison of neuroinvasive (NY99) vs. non-neuroinvasive strain (Eg101) of WNV further demonstrated dramatic differences between induction of specific CAMs in HBMVE cells following infection.

Conclusion: Collectively, our results for the first time demonstrate how neuropathogenic strain of WNV modulates the interactions between BBB microvascular endothelial cells and leukocytes to gain entry into the brain. These data suggest that targeting CAM signaling may help control WNV neuroinvasion and associated neuropathology.

Poster Session - Board 042 / 35

A real-time PCR method to study relative dengue virus replication using oligo-dT primed cDNA

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Real-time PCR is a powerful technique used in quantifying mRNA. However, along with its highly sensitive nature comes the issue of handling introduced and inherent error. Data normalization therefore becomes a necessary yet difficult problem to address. In this study, a SYBR green real-time PCR method to quantify relative dengue virus (DENV) levels using oligo-dT primed cDNA is introduced. This method is based on the finding that oligo-dT can prime both DENV RNA and an endogenous reference gene from within one RNA sample allowing for an endogenous and internal control.

In relative DENV studies, random primers are often used to synthesize DENV cDNA as DENV RNA is known to lack a poly-A tail. Random primers can anneal non-specifically throughout the target RNA. Additionally, all RNAs, including the highly abundant rRNA and tRNA will be transcribed. Oligo-dT primers anneal to poly-A tails to yield a more specific reaction than random primers and provide the best option to obtain a cDNA representation of mRNA. In this study, it is shown that oligo-dT can prime the RNA of all four DENV serotypes and the utilization of this cDNA in relative DENV studies may be advantageous over the use of random primed cDNA.

Breakout Session IIIC: Health Disparities / 36

Short and long term effectiveness of exercise, and mindfulness education in stress reduction in an American Indian population

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This study was conducted to assess whether mindfulness-based stress reduction (MBSR) combined with exercise would reduce stress more than MBSR alone on the Fort Peck Reservation in Montana. There are many stressors negatively affecting the health of the population and potentially shortening life expectancy and quality, yet little research on stress reduction has been done in this population.

Participants were randomly assigned to either an education group or exercise and education group. The participants were given pre and post-tests. The Derogatis Stress Profile (DSP) and Cohen Perceived Stress Test measured stress and the Mindfulness Self Efficacy Scale (MSES) Cayoun, B., Freestun J. (2004) measured mindfulness. Exercise and practices completed by subjects were logged weekly. Personal journals were kept by subjects to record thoughts, feelings, and practices. Journals were collected at the end session. At the last session a satisfaction and practices survey using a Lickert scale was administered.

All participants met for two hours a week for ten weeks for stress education classes. Education consisted of meditation and yoga using A Mindfulness-Based Stress Reduction Workbook , Bob Stahl, PH.D and Elisha Goldstein, PH.D. This workbook is based on the Mindfulness Based Stress Reduction program developed by Jon Kabat-Zin. Exercise and education participants exercised for three hours each week for ten weeks in addition to the MBSR classes. Exercise consisted of aerobic and resistance training supervised by exercise coaches at local wellness centers.

The research assistants contacted participants and recorded data collected to monitor the mindfulness study practices of participants during the intervention period.

Pre and post-test results were analyzed using the student t-test, $p = 0.05$.

Results showed no significant difference between the exercise and education group versus the education only group. Both groups did show a significant decrease in stress and increase in mindfulness. Journals entries indicated a gradual increase in understanding of MBSR and changes in stress responses as well as increased self awareness. Survey results indicated a high satisfaction with intervention, with most highly recommending it. Results from tests given1year later show no significant difference from post-test scores.

Our results indicate MBSR alone can reduce stress and create more mindful awareness in the lives of the population with a potential long term efficacy. Given the highly stressful environment an inexpensive and non-pharmacological treatment would be of great benefit to the community.

Poster Session - Board 086 / 37

Wyoming IDeA Networks for Biomedical Research Excellence (INBRE)

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The Wyoming IDeA Networks for Biomedical Excellence (INBRE) Program is funded by the National Institutes for Health Institute for General Medical Sciences (NIGMS). The ultimate goal of the NIGMS INBRE program is to promote the development, coordination, and sharing of research resources and expertise that will expand research opportunities and increase the number of competitive investigators in the IDeA-eligible states. To accomplish these goals Wyoming INBRE has established a number of programs designed to provide support to activities at all levels of higher education that enhance biomedically related research, training, education, and recruitment. Support for research is targeted at junior investigators conducting projects and programs that address health issues important to Wyoming residents that range from bench top research to clinical, translational, or community based investigations. Current areas of particular interest are cardiovascular health and Type 2 diabetes. Support is available for researchers through the Thematic Investigator Grant Program, Pilot Grant Program, Graduate Research Assistantship Program, Undergraduate Support Grant Program, and the UW- Community College Collaborative Grant Program. Wyoming INBRE also partners with other UW units to provide faculty start-up support for biomedically targeted faculty hires and for major equipment purchases to build UW's research infrastructure. To support biomedically related education and training several programs are available to faculty and students at UW and Wyoming Community Colleges. Support is provided to Wyoming Community College faculty to develop research projects on their campuses that engage students in the process of science and recruit them into biomedically related degree programs. The Outreach/INBRE Videoconference System is available for courses, seminars, and meetings for INBRE and other activities. Other programs include: Transfer Student Scholars Program that supports outstanding community college students transferring to the University to pursue baccalaureate degrees; Transition Course Program that supports development of distance delivered upper-level courses for students across Wyoming pursuing biomedical/health science related education; UW undergraduate student scholars program; Bioinformatics Summer Institute for students interested in pursuing advanced education and training in bioinformatics; and the INBRE Community College Videoconference Seminar Series that provides monthly seminars to community college faculty and students from UW, Community College and visiting scientists. Last, Wyoming INBRE is working with other western INBRE states to offer regional opportunities for research collaborations and educational opportunities for students and faculty. Support is available for researchers and students pursuing collaborative projects and educational opportunities with INBRE colleagues in Alaska, Idaho, Montana, Nevada, New Mexico, Montana and the University of Washington Institute for Translational Sciences (ITHS), University of New Mexico Clinical and Translational Sciences Program (CTSA), and the University of Colorado Medical Center CTSA.

Breakout Session IIA: Cardiovascular / 38

Physiology of cardiac hypertrophy in severely iron deficient rats using pressure-volume loops

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Background:
Cardiac hypertrophy, enlargement of the heart, can be adaptive or pathological. Aerobic athletes adapt to increase cardiac output with healthy muscle and larger ventricular chambers. Congestive heart failure decreases cardiac output with fibrosis, but no chamber enlargement, resulting in pathology. Iron deficiency (ID) causes cardiac hypertrophy. Although its physiology is unknown, studies have suggested chronic sympathetic nervous system (SNS) stimulation is involved. Short-term stimulation of the SNS is adaptive; chronic stimulation may be pathological. As little as two weeks of ID causes hypertrophy; by 12 weeks, ID hearts are apoptotic and dysfunctional. A transition from adaptive to pathological must occur between these times, which can be demonstrated physiologically.
Objective:
We hypothesized that four weeks of ID would result in failing cardiac function, measured using pressure-volume loops.
Methods:
We placed 8 rats on control or ID diets for 4 weeks, and then determined cardiac function in vivo using pressure-volume loops. We implanted catheters in both femoral veins (for drug infusion), a jugular vein (for saline calibration), and inserted a pressure-volume micro-catheter into the left ventricle via the right carotid artery. We occluded the inferior vena cava for load-independent measurement of contractility, and infused hypertonic saline via jugular vein for parallel conductance calibration. We used dopamine (beta-agonist) and atenolol (beta-antagonist) to assess cardiac responsiveness to SNS stimulation and blockade, respectively. We heparinized, decapitated, and collected blood for conductance-volume calibration, hematocrits, and plasma catecholamine analysis by high performance liquid chromatography (HPLC). We dissected hearts for morphometric and heart catecholamine analysis.
Results:
After four weeks of ID, three out of four ID rats showed significant increases in cardiac output, stroke volume, end diastolic volume, and ejection fraction (one ID rat demonstrated dramatic cardiac dysfunction, and was excluded from further analysis). Mean ejection fraction of ID rats was 93 percent, nearly twice that of control rats. Contractility and afterload were not significantly different, so the significant increase in stroke volume with ID was due entirely to ejection fraction changes. Since heart rate was also not different between the groups, the significant increase in cardiac output with ID can be attributed to the increase in ejection fraction.
Conclusion:
Our hypothesis that four weeks of ID would result in failing cardiac function, was not supported. Instead, we found three of the four ID rats were in an adaptive state, while one appeared to be failing. Since contractility and heart rate were not significantly increased, we conclude that the physiological changes were not due to chronic SNS activation, but they are consistent with the Frank-Starling Law of the Heart. Work is underway to examine cardiac function with longer periods of ID.

Breakout Session IC: Infectious Diseases - Parasites & Vectors / 39

Coccidia Presence in Wild Avian Communities of the Big Horn Basin

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Coccidia are single-celled, intracellular, intestinal parasites infecting a variety of animals including wild birds. Eimeria and Isospora, with multiple species, contribute to the disease commonly known as coccidiosis, one of the most prevalent diseases in animals in North America. The purpose of this study was to assess the presence, diversity, and ecology of coccidia in the wild avian communities of the Big Horn Basin, Wyoming.

The presence of coccidia was ascertained through collection of birds' (predominantly passerines) fecal materials. To sample the feces, we placed ten bird feeders baited with black oil sunflower seeds and millet/milo/cracked corn mix. Plastic sheets were placed under each bird feeder, and samples were collected twice daily: 11:30 MDT and 16:30 MDT. We collected 220 samples in total. The samples were placed into tubes with a 2.5% solution of potassium dichromate and then transferred into Petri dishes. After 10 days, samples were floated in 30 ml centrifuge tubes, with wet mounts prepared of the least dense supernatant. Each slide was scanned at 100x-1000x for the presence of coccidia. No coccidia were encountered in the first 64 samples, leading to an investigation of alternate protocol for the identification of coccidia from small (~ 0.15g) passerine fecal samples. The alternate protocol took into consideration the sample's weight. Hence, the samples were processed in proportion 1: 2 to sugar solution in 3 ml centrifuge tubes with the addition of two drops of Schiff's dye reagent. The remaining 156 samples were processed through the second protocol; 35 of these samples slides contained coccidia oocysts. Chi-square analysis of coccidian presence and type showed distinct patterns by protocol used as well as sampling location (χ^2=14.448, df=1, p<0.001). The second (i.e., original to this work) protocol was significantly more efficacious in comparison to the first protocol. Regarding the species distribution, Isospora spp. were mostly present at the Northwest College campus area whereas Eimeria spp. were mostly present off-campus (χ^2=27.935, df=2, p<0.001).

We confirmed the presence of coccidia among wild avian communities of the Big Horn Basin. Moreover, it showed interesting patterns in coccidia species distribution, leading to the conclusion that even in small areas, there is significant heterogeneity in coccidia communities. Further research may elucidate the importance of avian species, season, habitat, epidemiology, or factors yet to be determined, in predicting coccidia presence and type.

Poster Session - Board 085 / 40

Nevada's COBRE in Cell Biology: Establishing a Center with Focus on Signaling across Membranes

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Background. Signaling is important for normal intracellular and cell-to-cell communication, and dysfunction of signaling pathways can cause a variety of human diseases. At our university, several labs have expertise in signaling; however, they lacked a formal and coherent organizational structure and are separated by colleges and campus location.
Objective. We seek to integrate and enhance collaborations in cell biology through the NIH-funded "Center of Biomedical Research Excellence" (COBRE) mechanism. Our primary goal is to enhance biomedical research capacity in cellular and molecular biology of signaling at the University of Nevada.
Methods. Our center supports the work of promising junior investigators by implementing a mentoring and faculty development program and by strategic hiring of talent from outside Nevada. We design new core support, purchase new equipment, and help support service contracts for maintenance of sensitive equipment. Our lab, core and office spaces have been renovated and specifically tailored to the needs of our junior investigators. Throughout these activities, we foster collaborations and networking between faculty from the College of Science and the School of Medicine, and strive to enhance external funding.
Results. Now at the end of our second year of funding, we have hired three new junior faculty members into our program. Approximately 8,000 square feet of lab, core facility and office space have been renovated; our major purchases include a new Leica confocal microscope and a Bioscience Seahorse analyzer for studies of oxidative metabolism. We have created three new core facilities, and collaborations among our COBRE members resulted in exciting new grant applications. Center personnel have already received regional and international honors and awards, such as being selected to deliver the distinguished College of Science Christmas lecture at the University of Nevada (Tom Kidd, 2012) and receiving the prestigious "next generation award" of the Society for Neuroscience for education/outreach (Amy Altick, 2012). Some of our center's work, on signaling in Drosophila, was highlighted in a recent Cell Report article. This work from Tom Kidd's lab shows that axon pathfinding surprisingly utilizes apoptotic signaling pathways. Also, new grant applications by the Berninsone lab seek to develop treatments for parasitic worm disease by exploiting glycosylation of signaling proteins that are unique to nematodes. The research of our three new hires focuses on various signaling aspects of neurodevelopmental and neurodegenerative disease.
Conclusion. Integration of research efforts between colleges and across campus is challenging, but possible. COBRE funds were instrumental in the hire of three new faculty members with complementary expertise in cell biology, while renovation of core and lab facilities and purchases of novel equipment provide the supporting infrastructure for enhanced and exciting new biomedical research in cell biology at the University of Nevada, Reno.

Breakout Session IA: Neuroscience / 41

Integrated analysis of microRNAs induced by West Nile virus infection and their disease related targets in mouse brain

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Background: MicroRNAs (miRNAs) are small noncoding RNA molecules, which regulate gene expression in a wide range of physiological and pathological conditions. Recent studies have demonstrated that cellular miRNAs play important role in the pathogenesis of various viral infections and neurodegenerative disorders. West Nile virus (WNV), a mosquito-borne flavivirus, is a leading cause of arboviral encephalitis in the United States. Inflammation in the brain is a major hallmark of West Nile virus-associated encephalitis in mice.

Objective: The objective of this study is to determine the role of cellular miRNAs in WNV-induced neuroinflammation. Herein, we evaluated the expression profile of miRNAs and their correlation with genes involved in inflammatory pathways in WNV-infected mouse brain.

Methods: C57BL/6J mice were infected with 10 PFU of WNV or PBS (mock) and brains were harvested at day 8 after infection for miRNAs expression analysis using quantitative real-time polymerase chain reaction (qRT-PCR) based miRNA PCR Array. Similarly, mRNA expression of key pro- and anti-inflammatory molecules in the same brains were also analyzed using mRNA PCR arrays to examine whether the differentially expressed miRNAs could regulate their target genes.

Results: A total of 528 miRNAs were examined. One hundred thirty-nine miRNAs were significantly differentially expressed in WNV-infected mouse brain. Thirty-six miRNAs were significantly up-regulated, and 103 were down-regulated by > 2-fold in infected brains when compared to mock brains. Ingenuity pathway analysis demonstrated that these miRNAs and their targeted genes are involved in pathways related to inflammatory response, neurological disease, immune response and cell death. Moreover, using same mice brain samples, we demonstrated an inverse correlation between the miRNAs expression and their target mRNA genes. Our data further demonstrate that a single miRNA can target multiple genes involving cytokines, chemokines, transcription factors and apoptotic genes, which belong to different signaling pathways that play a critical role in WNV neuropathogenesis.

Conclusion: This study for the first time demonstrates that WNV infection causes modulation of miRNAs expression in the mouse brain, which may control gene expression of pathways that are important for WNV disease pathogenesis and can be targeted in the future to develop therapeutics for the management of WNV disease.

Funding: Institutional funds and grant (P20GM103516) from the Centers of Biomedical Research Excellence (COBRE), National Institute of General Medical Sciences, National Institutes of Health. We thank Dr. Gordon Okimoto and Mr. Mike Loomis of University of Hawaii Cancer Center and COBRE Bioinformatics Core for assistance with Ingenuity analysis.

Poster Session - Board 047 / 42

Type 2 diabetes in mice impairs immune cell infiltration and neuroinflammation after West Nile virus infection

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Background: Diabetes is a significant risk factor for developing West Nile virus (WNV)-associated encephalitis (WNVE) in humans, the leading cause of arboviral encephalitis in the United States. Using a diabetic mouse model, db/db, we recently demonstrated that diabetes enhanced WNV replication and susceptibility of mice to WNVE. Increased mortality in db/db mice was accompanied with enhanced WNV replication in the brain. However the underlying mechanisms associated with increased brain viral load and disease severity in db/db mice are unknown.

Objective: The objective of this study was to examine immunological and pathological events in the brain of wild-type (WT) and db/db mice after WNV infection in order to understand neuropathogenesis of WNV infection in diabetics.

Methods: Nine-week old C57BL/6 WT and db/db mice were infected with WNV and leukocyte infiltration, expression of cell adhesion molecules (CAM), neuroinflammatory responses, activation of astrocytes and neuronal death were determined by qRT-PCR, WB, immunohistochemistry, ELISA, Luminex assay, PCR arrays, flow cytometry and TUNEL assay.

Results: Our data demonstrate that infiltration of CD45+ leukocytes and CD8+ T cells were significantly reduced in the brain of db/db mice when compared to the WT mice. Further, we demonstrate that decreased migration of leukocytes was correlated with attenuated expression of CAM such as E-selectin and ICAM-1 in the brain of db/db mice. Despite reduced immune cell infiltration, WNV infection in db/db mice was associated with enhanced inflammatory response in the brain characterized by significantly high levels of multiple cytokines and chemokines, which correlated with increased levels of WNV in the brain of db/db mice. Elevated levels of cytokines also correlated with increased astrocytes activation and neuronal damage in the brain of db/db mice.

Conclusion: These data suggest that reduced leukocytes recruitment in the brain, in part, due to lower levels of CAM, results in failure to clear WNV infection leading to increased neuroinflammation, which mediates increased neuronal death and mortality in db/db mice. This is the first study to elucidate neuropathogenesis of WNV infection in a diabetic mouse model and can be used to develop adjunct therapies to manage WNVE in diabetics.

Funding: Institutional funds and grant (P20GM103516) from the Centers of Biomedical Research Excellence, National Institute of General Medical Sciences, National Institutes of Health.

Breakout Session IA: Neuroscience / 43

Development of a Human Polyomavirus JC Infection Model Using Humanized NSG Mice

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Background: Progressive multifocal leukoencephalopathy (PML), caused by the human polyomavirus JC (JCV), remains an important cause of morbidity and mortality among immunocompromised patients including patients with AIDS. While archetype JCV is widespread and circulates in healthy human population, only the rearranged type JCV (Mad-1) causes PML. JCV can only infect humans and data on primary site(s) of infection, latency, and the rearrangement of archetype JCV to rearranged type JCV during immunosuppression is limited due to the lack of an animal model. Recently, a novel murine model, 'humanized NOD scid gamma (NSG) mice', has been employed to study human-specific pathogens. NSG mice are immunodeficient mice reconstituted with human hematopoietic stem cells. Objective: Herein, we infected NSG mice with JCV and evaluated the course of ensuing infection in a quest to develop and characterize an animal model to study the pathogenesis of JCV infection. Methods: Prior to infection, human immune cells engrafted in NSG mice was confirmed by flow cytometry analysis. NSG mice were infected with either archetype JCV or rearranged JCV by intravenous (IV) injection. Blood and urine were collected at days 3, 5, 7, 14, 21, and 28 after infection and JCV virus DNA and T antigen protein in NSG mice was detected by real-time PCR (qPCR) and flow cytometry, respectively. Results: Our data demonstrate that NSG mice have at least >50% human cells as determined by the percentage of human CD45+ cells in the peripheral blood by flow cytometry. Both archetype JCV and rearranged JCV productively infected NSG mice. JCV T antigen DNA was detected as early as day three after infection in urine of mice, which peaked at day 7 after infection. JCV T antigen DNA was first detected in the blood of NSG mice on day seven after infection. JCV T antigen protein was also detected in the blood on day 7 after infection as measured by flow cytometry. JCV T antigen DNA was detected in the urine and blood of NSG mice up to two weeks after infection. Conclusions: This is the first study to demonstrate archetype and rearranged JCV infection in humanized NSG mice. Humanized NSG mice infection of JCV provides an important animal model for JCV primary infection. Future research is focused on the routes of primary infection and mechanisms of reactivation after HIV co-infection.

Poster Session - Board 002 / 44

Quipazine-induced air-stepping in the perinatal rat: Is it locomotion?

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BACKGROUND: The serotonergic agonist quipazine previously has been shown to be effective in eliciting robust forelimb and hindlimb stepping in rat fetuses and newborns (e.g., rat pups show about 800 alternated hindlimb steps during a 30-minute period); however, whether or not quipazine-induced stepping represents actual locomotion in perinatal rats is unclear. OBJECTIVE: The purpose of this study was to determine if quipazine could facilitate postural control and patterns of locomotion in one-day old rats. METHODS: Subjects received a 0.05 mL intraperitoneal injection of either 3.0 mg/kg quipazine or saline (vehicle control) and then were placed on a clear, plastic surface where locomotor behavior was recorded for a 45-minute test session. RESULTS: A significant effect of quipazine on locomotor behavior and postural control was found. Compared to controls, quipazine-treated pups showed significantly more bouts and longer durations of pivoting, crawling, limb activity, and head elevation. They also were more likely to show quadrupedal walking. CONCLUSION: Thus quipazine does induce postural control and patterns of locomotion in freely moving newborn rats, suggesting that quipazine-induced air-stepping is a useful model for studying the neurobehavioral development of locomotion. [NIH grant #1R15HD062980-01 to MRB, the INBRE Program, NIH Grant Nos. P20 RR016454 (NCRR) and P20 GM103408 (NIGMS) and ISU HSSRC grant to HES]

Poster Session - Board 022 / 45

Adiponectin deficiency accentuates second hand cigarette smoke-induced cardiac dysfunction: role of autophagy, ROS and apoptosis.

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Background: Exposure to second hand cigarette smoke is deemed an independent risk factor for cardiovascular disease. Second hand smoke is responsible for an estimated 46,000 cardiovascular deaths in non-smokers each year in the United States. Although the association between smoke exposure and cardiovascular disease has been well established, the underlying mechanisms are still poorly understood due to the lack of appropriate experimental models. Adiponectin (APN), an adipose-derived adipokine, offers cardioprotective effects although the precise mechanism behind APN-exerted beneficial action is unclear.
Objective: This study was designed to use a mouse model of exposure to cigarette smoke, a surrogate of environmental tobacco smoke, to evaluate the impact of cardiac-specific deletion of adiponectin on myocardial geometry, contractile and intracellular Ca2+ properties, autophagy and apoptosis following second hand smoke exposure.
Methods: Adult C57BL/6 wild-type and APN deficient mice were placed in a cage exposed to 1 cigarette's smoke for 1 hr/day for 40 days. Echocardiographic, cardiomyocyte contractile, intracellular Ca2+ properties and reactive oxygen species (ROS) were examined. Western blot was performed to examine protein markers of apoptosis and autophagy pathways.
Results: Smoke exposure reduced myocardial and cardiomyocyte contractile function, disrupted intracellular Ca2+ homeostasis, provoked ROS accumulation and apoptosis, the effects of which were accentuated by APN deficiency. Furthermore, ANP deficiency augmented second hand smoke-induced increases in AMPK phosphorylation, ULK1 phosphorylation and the autophagy adaptor p62, Atg5 and ratio of LC3II to LC3I.
Conclusions: Our results revealed that APN deficiency aggravates second hand cigarette smoke- induced ROS accumulation, apoptosis and cardiac dysfunction through autophagy induction and interrupted autophagosome maturation.

Poster Session - Board 067 / 46

Stone Child College Rural Health Initiative

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Stone Child College's Rural Health Initiative was undertaken to address the existing health disparities between the Rocky Boy Chippewa-Cree Tribe and the US, All Races in the areas of diabetes, obesity, diseases of the heart, chronic obstructive pulmonary disease, influenza, pneumonia, cancer, suicide, mental health, and substance abuse. Stone Child College developed a partnership with the Chippewa Cree Wellness Coalition, Rocky Mountain Tribal Epidemiology Center, Montana State Suicide Prevention Program, KHEW Radio Station, local schools, local health center public health nursing program, Benefis Health Care System and numerous other entities to assist with this project. The project tracked where we have been, historically, and where we want to go to achieve a healthy community. A series of 9 modules were presented, 2 surveys were administered, a strategic plan was developed, and a review of tribal health codes was conducted to address priority areas identified by the community. Our journey has moved us to a point where we can apply for public health accreditation. We would like to share our journey with other American Indian tribes in the states of Minnesota, North Dakota, South Dakota, Montana and Washington.

Breakout Session IIB: Immunology & Tumor Suppression / 47

Dengue immune status of host affects the binding avidity and neutralizing potency of cross-reactive anti-envelope antibodies

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Background: The envelope (E) protein of dengue virus (DENV) is the major target of neutralizing antibodies (Abs) and vaccine development. Previous studies of human sera following DENV infection have shown a significant proportion of anti-E Abs were cross-reactive to all four DENV serotypes and to one or more other flaviviruses, known as flavivirus group-reactive (GR). Studies of mouse anti-E monoclonal antibodies (mAbs) reported that GR mAbs were weakly or non-neutralizing compared with type-specific mAbs. The epitopes, binding avidity and the relationship to neutralization potency of human GR mAbs remain largely unknown.

Objective: In this study, we investigated the epitopes, binding avidity, neutralization potency and mechanism of neutralization of 32 human GR mAbs, including 12 derived from patients with primary DENV infection (kindly provided by Drs. S. Kliks and S. Halstead) and 20 derived from patients with secondary DENV infection (kindly provided by Drs. J. Mongkolsapaya and G. Screaton).

Methods: A previously described dot blot assay involving 67 alanine mutants of surface-exposed E residues and capture-ELISA using virus-like particles were used to identify the epitopes on DENV E protein recognized by mAbs. Virion-capture ELISA and focus-reduction neutralization test were used to determine the binding avidity and neutralization potency, respectively.

Results: The epitopes involved either fusion loop (FL) residues in E protein domain II only or both FL and bc loop residues in domain II; these residues were highly conserved by different flaviviruses and absolutely conserved by the four DENV serotypes. A linear relationship was found between the neutralization potency and binding avidity. The neutralization potency and binding avidity of GR mAbs derived from secondary DENV infections were significantly higher than those derived from primary infections. GR mAbs derived from primary DENV infection primarily block attachment, whereas those derived from secondary infection block DENV at post-attachment. Analysis of the repertoire of anti-E mAbs derived from patients with primary DENV infection revealed that the majority were GR, low avidity and weakly neutralizing, whereas those from secondary DENV infection were primarily GR, high avidity and potent neutralizing. These observations suggest the weakly neutralizing GR anti-E Abs generated from primary DENV infection become potent neutralizing against four serotypes after secondary infection.

Conclusion: The finding that dengue immune status of host affects the quality of cross-reactive Abs generated may have implications for new strategies of DENV vaccine development.

Breakout Session IIIA: Infectious Diseases - Viruses / 48

Influenza A virus activates mast cells to enhance viral-induced pathology

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Background and Objective: Influenza A virus (IAV) is a seasonal respiratory pathogen that causes significant morbidity in the young and elderly populations, and has a dramatic economic impact. Additionally, IAV has the potential to cause global pandemics, which have significantly greater and more broad morbidity and mortality. This appears to be the result of an excessive immune response causing severe lung pathology. The lung is protected from pathogens by alveolar epithelial cells, tissue resident alveolar macrophages, dendritic cells, and mast cells; however, the role of mast cells during protective and pathological IAV infection has been under-explored.

Methods: Mast cell knock-in mice were used to demonstrate their in vivo relevance during IAV respiratory infection. Additionally, an in vitro co-culture assay was used to dissect the mechanism(s) behind IAV-induced mast cell activation.

Results: Both A/WSN/33 (WSN) and A/PR/8/34 (PR8) influenza viruses cause significant immunopathology in C57BL/6 mice, but only WSN induced pathology that was mast cell dependent. Using in vitro-derived bone marrow cultured mast cells (BMCMC), we found that WSN, but not PR8, directly activated BMCMC to degranulate and produce leukotrienes, inflammatory cytokines, and anti-viral chemokines. Moreover, human H1N1, H3N2, and influenza B virus isolates could activate both murine BMCMC and the human mast cell line HMC-1 in vitro, suggesting this pathway could play a role during human infections. Mast cells could be infected by IAV, which was dependent on the viral hemagglutinin's specificity for a2,6-linked sialic acids. Cytokine and chemokine production from BMCMC occurs in a RIG-I/MAVS-dependent fashion; however, reconstitution of mast cell deficient mice with RIG-I-/- BMCMC generated lung pathology similar to wild type BMCMC, implying that mast cell degranulation, rather than production of cytokines, causes WSN induced lung pathology. Conversely, mast cell degranulation was induced through a RIG-I/MAVS-independent mechanism. Using recombinant WSN strains, we found an association between binding of the WSN hemagglutinin to a2,6-sialic acids and its activation by plasmin.

Discussion and Conclusions: We have identified a unique inflammatory cascade dependent on mast cells which could be therapeutically targeted to limit morbidity following infection with influenza virus.

This work is supported by NIH grant P20 RR103500, K22 AI091647, and R01 AI103353.

Poster Session - Board 071 / 49

Diet-Induced Obesity Leads To Excess Lung Inflammation After Asbestos Exposure

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Background: Asbestos exposure can lead to a number of physiological consequences including chronic inflammation, asbestosis, pleural plaques, and mesothelioma. It is well known that individuals differ in their responses to environmental exposures to particles, including asbestos. Although genetics can account for some of these differences, it cannot fully explain individual responses to asbestos exposure. One factor that has been associated in epidemiological studies with differences in severity of asbestos-related disease is obesity. Furthermore, body mass index has been correlated with some diseases (viz., pleural plaques and pleural thickening) in the case of Libby amphibole-contaminated vermiculite exposure. Asbestos exposure leads to activation of the NLRP3 inflammasome, a protein complex that leads to release of the pro-inflammatory cytokine IL-1β. Obesity has been shown to lead to excess inflammasome activity in several other organ systems.

Objective: Using a murine model of diet-induced obesity we wanted to determine if obese mice showed a greater inflammatory response, including increased inflammasome activation in alveolar macrophages, to asbestos exposure compared to non-obese mice and if a chronic exposure model leads to greater levels of pathology in the lung, i.e tissue fibrosis.

Methods: C57Bl/6 mice were put on a high fat diet for 12 weeks until they were 25% heavier than mice on the control diet. Mice were split into three groups. Alveolar macrophages were collected from the first group of mice and exposed to asbestos for 24 hr to determine if they showed indicators of increased levels of inflammasome activation by measuring IL-1β release. The second group of mice were exposed to asbestos for 24 hr and cytokine production in the lavage fluid was determined as well cell differentials to measure granulocyte influx. The third group of mice underwent a chronic asbestos exposure for 8 weeks. Lung pathology was determined by staining the tissue in order to determine the level of fibrosis development.

Results: We found that diet-induced obese mice showed greater levels of inflammasome activation via increased levels of IL-1β production in alveolar macrophages after asbestos exposure when compared to non-obese mice. Additionally, obese mice had greater levels of inflammatory cytokines (MCP-1 and TNF-α) and granulocyte influx in the lungs after a 24 hr asbestos exposure. After an 8-week chronic exposure to asbestos obese mice showed trends towards greater levels of fibrosis in the lungs.

Conclusion: Diet induced obesity causes greater levels of inflammation after asbestos exposure in the lungs due, at least in part, to excess inflammasome activation. This increased level of inflammation leads to a trend for greater levels of tissue fibrosis after long-term exposure.

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Poster Session - Board 084 / 50

Molecular and Cellular Immunology Core at the Pacific Center for Emerging Infectious Diseases Research

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Supported in part by the COBRE-Pacific Center for Emerging Infectious Diseases Research (http://pceidr.jabsom.hawaii.edu) since 2003, the Molecular and Cellular Immunology (MCI) Core has grown steadily in scientific mission and scope. Currently, the Core has a user base of more than 40 principal investigators representing diverse research disciplines. The overall tripartite mission of the MCI Core is to provide individualized immunology support services; research and development on immunological techniques and assays; and education and training in molecular and cellular immunology. Operating as a fee-for-service facility, the MCI Core is equipped with BD FACSAria, BD FACSCalibur, GuavaEAsyCytePlus, Luminex 200, Beckman-Coulter ViCell, CTL-ImmunoSpot® S5 Core Analyzer, Bio-Rad and BioTek ELx808 ELISA readers and washers, and Faxitron compact 43855D X-ray Irradiation System. Emphasis is also placed on developing new and customized immunological assays, as well as applying innovative uses of core instruments for non-immunological investigations. In addition, regularly scheduled training workshops are held to enrich the educational and mentoring experience of COBRE investigators and other faculty members and students throughout the university and across the research community at large. As the sole resource of flow cytometry, cell sorting and state-of-the- art immunological services for bioscience researchers in the westernmost IDeA state, the MCI Core is an excellent example of how investments made to improve the research infrastructure and environment and to maximize access to and utilization of specialized instrumentations can be transformative, in terms of increasing research productivity, accelerating scientific innovation, improving grant competitiveness, as well as fostering trans-disciplinary partnerships and translational research collaborations at the University of Hawai'i for the State of Hawai'i, and beyond.

Breakout Session IIIA: Infectious Diseases - Viruses / 51

A CRISPR immune response to viruses that infect bacteria

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Viruses that infect bacteria are the most diverse and abundant biological agents on the planet. In response to these pervasive viral predators, bacteria have evolved sophisticated adaptive immune systems that rely on Clustered regularly interspaced short palindromic repeats (CRISPRs) and their associated genes (cas). Using a combination of biophysical and structural techniques we aim to determine the mechanisms of CRISPR RNA-guided detection and destruction of invading DNA. Our results indicate that CRISPR RNA-guided surveillance complexes assemble into large helical nucleoprotein filaments that enhance target sequence hybridization and that target binding triggers a conformational change that may recruit a transacting nucleases. These emerging mechanistic insight are being used to repurpose CRISPR systems for new applications biomedical and biotechnical sciences.

Poster Session - Board 068 / 52

The impact of Adverse Childhood Experiences (ACEs) on behavioral health outcomes among female juvenile offenders

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Background:
While adolescence is a critical developmental period linking Adverse Childhood Experiences (ACEs) to negative adult health outcomes, few studies have screened for ACEs during adolescence or explored the cumulative effect of ACEs on adolescent behavioral health outcomes.

Objective:
A recent pilot project used existing data to assess the impact of ACEs on behavioral health outcomes (substance abuse and emotional distress) among female juvenile offenders.

Methods:
305 female adolescents who received sanctions to probation (with or without detainment) completed audio computer-assisted self-interviews. Validated measures were used to assess substance abuse (CRAFFT), psychological distress (BSI-18), and eight adverse childhood events (physical abuse, sexual abuse, physical neglect, father's incarceration, mother's incarceration, single parent custody, removal from home, running away from home). ACE scores were calculated by summing the total number of ACE categories (range 0-8). Logistic regression was used to assess the independent relationship between ACEs and substance abuse as well as emotional distress.

Results:
Almost half (49%) of participants reported 4 or more ACEs. After controlling for age, race, and detention, there remained a strong dose response relationship between the number of ACEs reported and substance abuse [1-2 ACEs (AOR=5.78; 95% CI 1.81, 18.52), 2-3 ACEs (AOR=6.87; 95% CI 2.19, 21.57) and 4-5 ACEs (AOR=20.23; 5.70, 71.85)]. ACEs were also associated with emotional distress [1-2 ACEs (AOR=3.56; 95% CI 0.64, 19.70), 2-3 ACEs (AOR=7.48; 95% CI 1.38, 40.54) and 4-5 ACEs (AOR=8.76; 95% CI 1.58, 48.57)].

Conclusion:
The pilot project supported the feasibility and potential impact of ACEs on substance abuse and emotional distress among females involved with the juvenile justice system. The Nevada INBRE award will support the development of a more complete adolescent ACE screening instrument and the assessment of the cumulative effects of ACEs on a wide range of behavioral health outcomes among both male and female juvenile offenders.

Poster Session - Board 048 / 53

Luminex-based Microsphere Immunoassay for Rapid and Sensitive Detection of Acute and Recent Dengue Virus Infection during Hawaii 2011 Dengue Outbreak

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Background:
Dengue virus (DENV), an emerging flavivirus is a significant global health problem. Dengue outbreak occurred in Hawaii in 2001 and 2011, with 153 and four cases, respectively. There is a need for improved dengue diagnosis, which is critical for effective patient management. Recently Luminex-based assays have been used for rapid and sensitive diagnosis of many viral and bacterial diseases. We developed Luminex-based DENV microsphere immunoassay (MIA) for detection of anti-DENV antibodies.

Objective:
The objective of this study was to compare the DENV MIA to 'gold standard' IgM antibody-capture (MAC)-ELISA and plaque reduction neutralization test (PRNT90) for detection of anti-DENV IgM and IgG antibodies, respectively, using serum samples obtained from DENV-infected patients or suspected cases in Hawaii 2011 dengue outbreak.

Methods:
DENV MIA was conducted using incubation of serum samples with microspheres coupled with DENV-2 antigen. Beads coupled with bovine serum albumin (BSA) were included to detect nonspecific binding. Total 42 samples were analyzed using DENV IgG MIA and PRNT, as well as DENV IgM MIA and MAC-ELISA.

Results:
Total 11 of 42 (26%) serum samples were positive for both DENV IgG MIA and PRNT90 and remaining samples were negative. DENV IgG MIA agreement, sensitivity and specificity was 100%. Total three of 42 (7%) serum samples were positive for DENV IgM MIA and MAC-ELISA. One sample was false positive by DENV IgM MIA, however it showed high BSA background or nonspecific binding. Remaining 38 samples were negative by DENV IgM MIA and MAC-ELISA. DENV IgM MIA agreement, sensitivity and specificity was 98, 100 and 97%, respectively.

Conclusion:
We conclude that DENV MIA is rapid and sensitive alternative to ELISA and PRNT assays. Inclusion of BSA beads in DENV IgM MIA provide confirmation for true-negativity of IgM false positive samples that result from nonspecific binding. DENV MIA can be effectively used for diagnosis of DENV infections during dengue outbreaks in Hawaii and worldwide.

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Breakout Session IIC: Infectious Diseases - Bacteria & Fungi / 54

Roles of LTB4, IL-1α and IL-1ß in leukocyte recruitment and activation during Aspergillus fumigatus infection

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Background and Objective: Aspergillus fumigatus is a mold that causes severe pulmonary infections in humans, such as invasive pulmonary aspergillosis (IPA). Currently, our knowledge of how A. fumigatus growth is controlled in the respiratory tract is limited. Phagocytic alveolar macrophages and the airway epithelial cells constitute the first lines of defense against inhaled A. fumigatus conidia; subsequently, neutrophils and inflammatory macrophages are sequentially recruited to the respiratory tract to control fungal growth and germination. But how neutrophils and macrophages are recruited to the respiratory tract after A. fumiguatus infection remains ill defined.

Methods: Pycard-, Ltb4r1- and Il1r1-deficient mice were used to determine the in vivo importance of IL-1 and leukotriene signaling during intratracheal Aspergillus fumigatus infection.

Results: A. fumigatus instillation in B6 mice induced the expression of LTB4 and IL-1ß within 24h and IL-1α within 48h. Interestingly, Ltb4r1- and Pycard-deficient mice were only mildly susceptible to A. fumigatus infection, while Il1r1-deficient mice were highly susceptible to A. fumigatus infection as measured by increases in fungal germination and lung tissue damage. Ltb4r1 is critical for early leukocyte recruitment, while IL-1α is crucial for continued leukocyte recruitment through its regulation of production of CXCL1. In contrast, the inflammasome (Pycard) and IL-1ß are essential for optimal activation of anti-fungal activity of BMDM.

Discussion and Conclusions: Taken together, our data reveal central, non-redundant roles for IL-1α and IL-1ß in controlling A. fumigatus infection in the lung; specifically, pulmonary leukocyte recruitment is regulated by an LTB4-> IL-1α->CXCL1 sequel, while IL-1ß is necessary for optimal activation of anti-fungal activity of those leukocytes.

This work was partly funded by NIH grant 5P20GM103500.

Poster Session - Board 079 / 55

Advancing collaborative biomedical research and promoting core research facilities through an open source laboratory informatics solution and semantic search application

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A vexing and chronic problem for basic and clinical scientists engaged in biomedical, socio-behavioral and translational research is the frequent difficulty in identifying and accessing research resources and core services, which while often available are virtually invisible within and across research institutions. In 2009, in an uncompromising effort to address this issue, a nine-institution consortium, comprising institutions in five IDeA-eligible states and Puerto Rico, was awarded a two-year grant, funded through the American Recovery and Reinvestment Act, to build a robust inventory tool and search application for unique shareable research resources, widely known as eagle-i. Building on this early success, a growing network of 26 institutions is now leveraging experiences gained and lessons learned during the initial stages of this project to significantly expand an already sizable inventory of research resources, thereby enhancing in the process research infrastructure at old and new participating institutions. This poster illustrates the breadth and scope of core facilities funded by the INBRE and COBRE programs at the University of Hawai'i at Mānoa, that are part of this federated repository of research resources and core services fully accessible through the eagle-i semantic search application. Deploying this revolutionary, open-source informatics tool across the entire Network of IDeA-funded Core Laboratories (NICL) would add tremendous value to an unprecedented effort to drive scientific discovery and accelerate innovation through resource sharing and collaboration within and beyond the IDeA community.

Poster Session - Board 046 / 56

West Nile virus infection-induced expression of TREM-1 modulates innate immune responses

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Background: Activation of Triggering Receptor Expressed on Myeloid cells 1 (TREM-1), an immunoreceptor expressed on immune cells, results in a cascade of inflammatory effects including cytokine production. In addition, TREM-1 modulates signaling cascades of TLRs and NLRs. Engagement of the TREM-1 receptor via the adaptor protein DAP12 activates downstream kinases and mobilizes intracellular calcium to produce pro-inflammatory cytokines. The soluble form of TREM-1 (sTREM-1) is suggested as an important predictor of specific inflammatory conditions. Innate immune responses are essential for the control of virus infection and dissemination of West Nile virus (WNV), a neurotropic flavivirus, which has emerged as a significant cause of viral encephalitis in humans in the U.S. However, the role of TREM family members in immunity to flaviviruses is unclear.

Objective: In this study we investigated the changes in the expression kinetics and activation of TREM family members (TREM1-4) following infection with flaviviruses.

Results: Expression of TREM-1 was markedly increased in dengue virus-infected THP-1 cells, which correlated with peak viral titers. Similarly, WNV infection led to a significant increase in the mRNA levels of TREM-1 in mouse embryonic fibroblasts (MEFs) and bone-marrow derived dendritic cells (BMDC) and macrophages (BMDM) at 48 and 72 hours after infection. In vivo characterization of multiple TREMs in WNV-infected mice demonstrated significant increase in the transcripts of TREM-1, -3 and -4 in the peritoneal cavity cells and brain at day 3 and 8 after infection respectively. We also observed a significant increase in the serum levels of sTREM-1 between days 2-3 after WNV infection. Further, activation of TREM-1 using an agonist antibody resulted in an increase in the innate immune markers such as IFN-α, TNF-α and IL-6, which correlated with reduced WNV replication in MEFs. On the other hand, inhibition of TREM-1 in BMDMs resulted in enhanced virus replication at 36 and 48 hours after WNV infection.

Conclusion: Thus, our results so far indicate a critical role of TREM-1 in the modulation of inflammatory innate immune responses to WNV and further studies are ongoing to characterize unexplored mechanisms of TREM-1 associated with the pathogenesis of flaviviruses.

Breakout Session IIC: Infectious Diseases - Bacteria & Fungi / 57

Determining the location and function of sterols in endomembrane-containing cells of the bacterium G. obscuriglobus

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Background
Gemmata obscuriglobus is a bacterium that possesses features unique for prokaryotes such as an extensive endomembrane network, endocytosis, and sterol biosynthesis. Among these features, the biosynthesis, cellular location, and functional role of sterols in G. obscuriglobus remains the least understood. Sterols have been shown to influence the physical characteristics of membranes and to be involved in endocytosis in eukaryotes. Therefore we hypothesized that sterols in G. obscuriglobus may fulfill a similar function(s), since the bacterium has both an extensive endomembrane network and endocytosis activity.
Objective
The objective of this study is to localize sterol deposition in G. obscuriglobus membranes and to understand the biological role of sterols in this bacterium.
Methods
To address the objectives of the study we have implemented the following methods: bioinformatic analysis of genes involved in sterol synthesis in G. obscuriglobus; localization of sterols through fluorescent and electron microscopy of Filipin-labeled sterols alongside cellular markers;. a genetic complementation test of G. obscuriglobus sterol genes using yeast, to understand the activity and function of these genes; a biochemical inhibition assay of sterol-synthesizing enzymes in G. obscuriglobus in order to determine the function of sterols.
Results
Genes encoding the sterol synthesis enzymes SQMO and OSC in G. obscuriglobus contain the necessary functional domains FAD and squalene cyclase, that are also found in eukaryotic homologues. We localized sterols to both intracellular and outer membranes using fluorescence microscopy. We also observed that inhibition of sterol biosynthesis by a sterol-binding drug did not affect the growth of G. obscuriglobus. Further investigations using the genetic complementation test are necessary to understand the functional role of sterols in G. obscuriglobus.
Conclusions
Given the prevalence of sterol biosynthesis in the eukaryotic, but not prokaryotic, world, our findings of sterol functions in G. obscuriglobus may lead to changes in our understanding of the development of complex multicellular life and evolution of biological pathways.

Poster Session - Board 031 / 58

RAW264.7 Macrophage as a Model of Foreign Body Inflammatory Response to Electrospun Biomaterials

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Background:
The host foreign body response (FBR) to biomaterials is an important initiator of wound healing, but excessive inflammation can be detrimental to the patient. There is a lack of efficient methods for evaluation of this response, and extensive in vivo characterizations are typically necessary. Here, we investigated an in vitro model involving macrophages, which are considered to be one of the primary mediators of FBR.

Objective:
The purpose of this study is to evaluate RAW264.7 macrophage inflammatory response to electrospun polycaprolactone (PCL) biomaterials in a controlled in vitro environment, and to interpret the effectiveness of this model for biocompatibility screening with respect to the FBR typically observed in vivo.

Methods:
PCL microfiber scaffolds were produced by electrospinning. RAW264.7 cells were grown on the scaffolds for six days. Supernatants were collected daily for ELISA detection of pro-inflammatory cytokines, Tumor Necrosis Factor (TNF)-α, Interleukin (IL)-1β, and IL-6. Throughout the culture period, cells were fixed and processed for confocal microscopy and SEM. RAW264.7 cells were grown on solvent cast PCL films and standard tissue-culture treated polystyrene (TCPS) for comparison. Lipopolysaccharide (LPS) treatment served as a positive control for cytokine production.

Results:
Cells adhered to the PCL fibers, infiltrated into the depth of the scaffold, maintained steady growth over six days, and fused to form Foreign Body Giant Cells (FBGC) by day three in vitro. Cytokine levels from cells grown on PCL scaffolds were negligible over six days. Cytokine levels from LPS-treated cells on PCL scaffolds were less than or equal to LPS-treated cells on TCPS.

Conclusions:
RAW264.7 cell attachment, infiltration, growth, morphology, and formation of FBGCs was qualitatively consistent with literature describing in vivo macrophage responses to electrospun polymers. Negligible cytokine production indicates electrospun PCL does not induce a pro-inflammatory response in RAW264.7 cells. Although presence of FBGCs is traditionally considered a hallmark of chronic inflammation, some studies suggest FBGC formation in response to non-phagocytosable biomaterials results in down-modulation of inflammation. Removal of macrophages actively secreting pro-inflammatory cytokines is a potential explanation for this phenomenon that warrants further investigation.
Although this in vitro macrophage model may serve as a method for initial biocompatibility screening of biomaterials, it cannot comprehensively substitute for in vivo assessment of FBR. In vivo models account for other factors influencing FBR, such as interactions among other cell types, inflammatory response to surgical implantation and the provisional matrix formed by blood-material interactions, extracellular matrix remodeling, and fibrous capsule formation. Therefore, the RAW264.7 model may be more appropriate for preliminary screenings, or when controlled, isolated environments are desired for investigation of macrophage-biomaterial interactions.
Work funded by NIH P30 GM103338 and R25 ES022866.

Breakout Session IIB: Immunology & Tumor Suppression / 59

Non-specific dsRNA-Mediated Innate Antiviral Immune Response in the Honey Bee

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Honey bees are essential pollinators of numerous agricultural crops. Since 2006, honey bee populations have suffered considerable annual losses that are partially attributed to Colony Collapse Disorder (CCD). CCD is an unexplained phenomenon that correlates with elevated incidence of pathogens, including RNA viruses. RNA viruses generate long dsRNA molecules during their replication cycle, which trigger mammalian innate immune responses and serve as substrates for virus-specific RNAi-mediated antiviral immunity in plants and insects. To investigate honey bee antiviral defense mechanisms, we developed an RNA virus infection model and examined the role of dsRNA in antiviral immunity. We discovered that administration of dsRNA, regardless of sequence specificity, reduced model virus infection. Our results suggest that dsRNA, a viral pathogen associated molecular pattern (VAMP), triggers an innate antiviral response that controls virus infection in honey bees. We are currently examining the mechanism and relative contribution of non-specific dsRNA-mediated and RNAi-mediated antiviral immunity in honey bees. Understanding these mechanisms is critical to assessing the role of viruses and/or immune deficiencies in colony losses and may lead to the development of strategies that enhance honey bee survival.

Breakout Session IIC: Infectious Diseases - Bacteria & Fungi / 60

Prokaryotic Single-Cell Transcriptome Reveals Novel Pathogenesis mechanisms and Intracellular Behavior of Burkholderia pseudomallei

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Background: B. pseudomallei (Bp) is the etiological agent of melioidosis, a serious global emerging infectious disease. Within the context of a host cell infection, this potential bioterrorism agent Bp infects various cell types by going through different stages: i) entry into endocytic vesicles, ii) escape from these vesicles into the cytoplasm, iii) intracellular replication, iv) polymerization of cytoplasmic host-cell actin, and v) catapulting themselves directly into neighboring cells through membrane protrusions and fusion to spread from cell-to-cell. In the spatial compartments of host-cells, there has been a lack of assignment as to which Bp genes are differentially expressed, as the expression of these genes are important in the pathogenic process of this bacterium.

Objective: Our long-term goal is to understand the molecular pathogenesis of Bp within the in vivo cellular environment. The objectives are to thoroughly identify in vivo expressed- and essential-genes and to characterize the expression of these genes with respect the spatial resolution during infection of eukaryotic cells. The central hypothesis is that Bp, as they encounter uniquely different intracellular environment and perform sequential steps in the infection process, will undergo differential gene-expression at each stage of cellular infection.

Methods and Results: To begin unraveling the complexity of a very large Bp genome (7.2 Mbp) and to understand the cellular pathogenesis of Bp, we have isolated Bp in different eukaryotic cell compartments to perform transcriptomic analysis on single bacterium. Using this single-cell transcriptomic technology, we have indentified several Bp genes important for novel virulence mechanisms, including genes important for attachment to host-cells, host-cell fusion and bacterial spread, host-cell cytoskeleton rearrangement, autophagy evasion, and others. We have created dozens of Bp mutants, which are attenuated in host-cell infection and many are attenuated in BALB/c mice.

Conclusions: This research have furthered knowledge into the molecular pathogenesis of Bp infection and will lead to vaccines and novel strategies for drug development, and more immediately, these attenuated Bp mutants could serve as live attenuate vaccine strains.

Poster Session - Board 043 / 61

Comparison of a New Commercially Available Enzyme Immunoassay and In-house MAC-ELISA for Detection of Anti-Dengue Virus IgM Antibodies

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Background:
Dengue (DENV), a mosquito-borne flavivirus infects an estimated 50 million people globally. Effective management of severe dengue disease can be augmented by rapid diagnosis during the acute stage of infection. In majority of dengue infections, IgM antibodies appear within 3 to 5 days following the onset of fever. However, in secondary infection, IgM antibody titers are usually lower as compared to primary infection. Several rapid diagnostic tests are commercially available for detection of anti-DENV IgM antibodies. It is important to evaluate the performance characteristics of these kits in terms of sensitivity and specificity for accurate diagnosis of primary and secondary dengue infections.

Objective:
The objective of this study was to evaluate the US Food and Drug Administration (FDA) approved InBios DENV IgM Capture ELISA in comparison with 'gold standard' in-house IgM antibody-capture (MAC)-ELISA for detection of anti-DENV IgM antibodies.

Methods:
We compared the InBios DENV IgM Capture ELISA with an in-house DENV MAC-ELISA using 79 clinical serum samples collected from Hawaii, Vietnam, Niue, Singapore and American Samoa. Sensitivity, specificity, percent agreement, 95% confidence intervals and kappa coefficients were determined as measures of agreement between InBios assay and in-house MAC-ELISA for detection of anti-DENV IgM antibodies.

Results:
We observed significant correlation ($r2 = 0.80$; $P < 0.0001$) between the InBios assay and MAC-ELISA for detection of anti-DENV IgM antibodies. Agreement, sensitivity and specificity of InBios assay were 94, 92 and 94%, respectively. In addition, the InBios assay showed near perfect agreement ($\kappa = 0.87$) to in-house MAC-ELISA. Out of 79 serum samples, five serum samples exhibited discordant results. Two serum samples from Hawaii and Vietnam, from patients with previous dengue infection were false-positive using InBios assay. However, absence of recent secondary DENV infection was confirmed for serum sample from Hawaii based on the lower DENV-specific CD8+ T cell proliferative response observed after stimulation with cognate DENV epitope as compared to recently dengue-infected patients.

Conclusion:
We conclude that the InBios DENV Detect IgM Capture ELISA can be used for rapid (~5 hr), sensitive and specific diagnosis of acute or recent dengue infections during dengue epidemics, compared to MAC-ELISA, which requires 2-3 days for completion. Moreover, data analysis of discordant samples suggests that positive as well as negative antigens are critical for interpretation of the test results as they can detect background nonspecific reactivity of serum samples and thus should be included in immunoassays.

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Poster Session - Board 065 / 62

The Effects of Radon on the Apsaalooke (Crow) Reservation in Montana

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Background: Radon is a radioactive gas that seeps through cracks in your home and foundation. It is odorless, colorless, and tasteless. Radon is caused by the natural breakdown of the earth's elements in water, soil and rock. Radon is released during the natural decay of uranium, which is found in most rock and soil. It's also the second leading cause of lung cancer after cigarette smoking. If Radon levels are higher than 4pCi/L the EPA recommends that your home be better ventilated or fixed. The average indoor Radon level is 1.3pCi/L and 4pCi/L for outdoor. The Radon Research Project at the Little Big Horn College is research that is being conducted in all seven districts located throughout the Crow reservation. These districts include Wyola-Mighty Few, Lodge Grass-Valley of the Chiefs, Reno, Black Lodge, Pryor, No Water, and Big Horn. Methods: The types of methods used to inform the public were brochures and surveys. We went door to door conducting the surveys. We also used short-term Energy Laboratories Radon Gas Test Kits to test the homes for Radon. Student researchers will setup Radon kits in homes with basements and dirt/gravel crawl spaces. Results: Pryor, St. Xavier, Crow Agency, Lodge Grass, Wyola communities, and off reservation tribal members have been given the survey in the initial stage of this project. Within the communities 28 surveys have been completed. A 100% have never heard of Radon, 100% have never tested their home for Radon. We have also tested 100 homes on the Apsaalooke reservation. The levels range from .5pCi/L to 1.4pCi/L. Conclusion: We have found radon levels higher in older model homes. For the summer of 2012 our results indicated high Radon levels in the Lodge Grass – Valley of the Chiefs district and low Radon levels in Big Horn.

Poster Session - Board 053 / 63

The Apàake Project at Little Big Horn College (West Nile Virus) on the Apsaalooke Reservation in MT.

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Purpose: The Apàake (WNV) project at Little Big Horn College collaborates with Fort Belknap College, Carroll College & Montana State University-Bozeman to determine the incidence of the WNV in the state of Montana. Methods: The five sites across the Apsaalooke (Crow) Indian Reservation will be monitoring and identifying species of mosquitoes in the region (Culex tarsalis & Culex Pipens). Results: The five Apàake Project trap sites collected the mosquitoes (Culex tarsalis & Culex Pipens) to determine the potential for West Nile Virus infected in animals, birds, & humans. Conclusion: Found that there are more Culex pipiens at Site 3: Fort Smith; and at Site 1: L.B.H.C.; Site 2: Lodge Grass; Site 4: Pryor; Site 5: Wyola had a higher Culex tarsalis.

Breakout Session IIIB: Cell & Molecular Biology / 64

Identification of Microbial Isolates for Culturing a Euglenid Protozoan

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Background: First described in 1674 by Leeuwenhoek, the Euglenids are a group of flagellated protozoa known for their rhodopsin eyespot, a contractile motility (metaboly), and their ability to feed phototrophically and/or organotrophically. The phototrophic Euglenids are of special interest evolutionarily because their chloroplasts are the result of an endosymbiotic uptake of green algae. Additionally, the phototrophic Euglenids synthesize a wide variety of polyunsaturated fatty acids, making them valuable in biochemical and nutritional studies.
Objective: A sample from Twin Lakes, Idaho yielded a Euglenid protozoan, G12, initially selected for its ability to grow in low light and its longevity on solid medium. Because G12 survived long-term storage in a mixed consortium, it was hypothesized that one or more of the co-cultured microorganisms supplied essential nutrients. The primary goal of this study was to isolate the G12-associated microorganisms and test their ability to enhance the growth of axenic G12.
Methods: Samples were streaked onto nutritious agar plates and non-green, semi-isolated colonies were subsequently streaked onto additional plates for further isolation. DNA from isolates served as template for 16S rRNA gene amplification PCR.
Results: It was found that at least eight different culturable microorganisms were present in the G12 mixed consortium. 16S rRNA gene sequencing revealed that a minimum of four bacterial genera were represented among the microbial isolates. Of those, three are known for their capacity for nitrogen-fixation. Additionally, preliminary experiments indicated that one of these nitrifying bacteria promotes growth of G12 when combined in a minimal medium under either high light or very low light conditions to restrict photosynthesis.
Conclusion: Since our growth studies indicate that G12 grows best under conditions that include a phototrophic source for nutrients as well as a microbial source, we conclude that G12 has the capacity for predation of smaller microorganisms. This trait could be especially beneficial in reducing microbial pollution if G12 were grown in open ponds.

Poster Session - Board 078 / 65

Creation of Leeward Community College's plant database and interface

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Background
Biodiversity informatics is an emerging field that integrates baseline information about taxonomy, pharmacology and resource conservation and use. A baseline plant diversity database of all trees, shrubs and native and dry land species collection in Leeward Community College campus was generated in a dynamic user platform.

Objective
This project was focused upon creating a working database and web-interface that would store and geospatially map out the plants present on Leeward Community College's 49-acre campus. The information to populate the database was collected through fieldwork, botany consultations and literature review.

Methods
Coordinates were collected by creating physical maps and referenced Google Earth. Plants were identified by their scientific names and then research was based upon these identifications. The database was made to be dynamic and remains open to handling ongoing research and additional changes to existing data or new plants that may arise. The web-interface was created through PHP: Hypertext Preprocessor (PHP), Hypertext Markup Language (HTML), Structured Query Language (SQL), JavaScript, Extensible Markup Language (XML) and used Google applications for mapping purposes.

Results
The interface provides access to the various data fields found in the database, in an intuitive graphical manner. Using a search list available on the interface, the user can select a plant attribute as a filter for the plants being displayed on the map. The database includes 1,096 individual plants with 238 unique species, 310 photographs and 197 external links. The database is designed to facilitate easy updates.

Conclusion
The research to populate the existing fields is on-going and once completed, will provide quantitative data including the number of native, endangered or poisonous plant forms found on the campus. Also, it will provide qualitative data through the medical uses and description tabs found on the user interface. The database is expected to be immensely valuable for future research, teaching and outreach activities of the college.

Poster Session - Board 088 / 66

Think Graduation: Chaminade's Curriculum Revitalization

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Background:
Chaminade University of Honolulu (CUH) is a federally designated Native Hawaiian serving institution that is positioned to lead in education, research and community development in the Pacific region.

Objective:
Strengthening and modernizing CUH's undergraduate curriculum by 2011 to position ourselves to be able to recruit and prepare the next generation of health practitioners, scientist and scholars.

Methods:
Since CUH's BS in forensic science had a flourishing program, the faculty embarked on a complete overhaul of the BS in biology and the design of a new BS in biochemistry during the 2010-2011 academic year. Our new curriculum design was driven by recommendations from agencies such as the Institute of Medicine, National Science Foundation, American Association of Medical Colleges and the Howard Hughes Medical Institute.

Results:
The new BS in biology and BS in biochemistry were approved by CUH's Academic Council in April 2010 and were entered into the university's catalog in Fall 2011. Key features were abolishing the BA in biology, reorganizing the biology BS into two available tracks (Cell and Molecular and Integrative and Organismal) and initiating the first ever BS in biochemistry at CUH. During this process, the faculty removed 30 obsolete courses from the catalog and created 36 new courses and electives. The faculty has also made a commitment to an inquiry based pedagogy and critical thinking wherever it can be incorporated into the curriculum.

Conclusion:
CUH offers a cutting edge biosciences curriculum that prepares students for graduate school in health professions, MS and PhD programs in biological and biochemical disciplines, work in government science agencies and non-government agencies, science policy-making and advocacy and biotechnology and pharmaceutical industries.

Breakout Session IIIB: Cell & Molecular Biology / 67

Helper Independent Self-inactivating Chimeric piggyBac Transposases for Genomic Targeting in Human Cells

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Efficient integration of functional genes is an essential prerequisite for successful gene delivery such as cell transfection, animal transgenesis, and gene therapy. Gene delivery strategies based on viral vectors are currently the most efficient. However, limited cargo capacity, host immune response, and the risk of insertional mutagenesis are limiting factors and of concern. Recently, several groups have used transposon-based approaches to deliver genes to a variety of cells. The piggyBac (pB) transposase in particular has been shown to be well suited for cell transfection and gene therapy approaches because of its flexibility for molecular modification, large cargo capacity, and high transposition activity. However, safety considerations regarding transposase gene insertions into host genomes have rarely been addressed. Here we report our results on engineering helper-independent pB plasmids. The single-plasmid gene delivery system carries both the pB transposase expression cassette as well as the transposon cargo flanked by terminal repeat element sequences. Improvements to the helper-independent structure were achieved by developing new plasmids in which the pB gene is rendered inactive after excision of the transposon from the plasmid. As a consequence, potentially negative effects that may develop by the persistence of an active pB gene post-transposition are eliminated.

An additional improvement has been the addition of the Gal4 DNA binding domain in order to enable pB to target transgenes to predetermined sites. Upstream activating sequence (UAS) Gal4 recognition sites harbored on recipient plasmids were preferentially targeted by the chimeric Gal4-pB transposase in human cells. To analyze the ability of these pB fusion proteins to target chromosomal locations, UAS sites were randomly integrated throughout the genome using the Sleeping Beauty transposase system. Both N- and C-terminal Gal4-pB fusion proteins but not native pB were capable of targeting transposition nearby these introduced sites. Genome-wide analysis revealed the ability of our fusion constructs to bias integration near endogenous Gal4 recognition sequences.

Recently, we attempted to retarget transposon insertions to a single genomic location by comparing a series of novel hyperactive pB constructs tethered to a custom transcription activator like effector (TALE) DNA-binding domain (DBD) designed to bind the first intron of the human CCR5 gene. Multiple targeting strategies were evaluated using combinations of both plasmid-DNA and transposase-protein relocalization to the target sequence. We demonstrated site-specific and user-defined transposition to the CCR5 genomic safe harbor and isolated single-copy clones harboring targeted integrations. This work provides a powerful approach to enhance the properties of the pB system for important applications such as genetic engineering and gene therapy.

Poster Session - Board 051 / 68

Phylogenetically divergent hantaviruses harbored by insectivorous bats in Côte d'Ivoire and Vietnam

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Background: Hantaviruses detected in multiple species of shrews and moles across four continents are genetically more diverse than hantaviruses harbored by rodents, suggesting that the host range of hantaviruses may be more extensive than previously imagined. In particular, mammals having shared ancestry with soricomorphs may have figured prominently in the diversification of hantaviruses. By virtue of their rich biodiversity, vast geographical distribution and demonstrated ability to host myriad viruses, bats are potential reservoirs of hantaviruses.
Objective: The goal of this study was to detect hantaviruses in insectivorous bats.
Methods: Either frozen, ethanol-fixed or RNAlater®-preserved tissues and fecal samples from 520 bats (representing six families, 26 genera and 53 species), captured in Asia, Africa and the Americas in 1981-2012, were analyzed for hantavirus RNA by RT-PCR.
Results: Following numerous failed attempts, hantavirus RNA was detected in tissues from two of 12 banana pipistrelles (Neoromicia nanus) (family Vespertilionidae), captured during June 2011 near Mouyassué village, in Côte d'Ivoire, and from five of 45 Pomona roundleaf bats (Hipposideros pomona) (family Hipposideridae), captured during May 1997 and March 1999 in Tuyên Quang and Quang Nam, respectively, in northern and central Vietnam. The RNA-dependent RNA polymerase-encoding L segment of the newfound hantaviruses, designated Mouyassué virus (MOYV) and Xuan Son virus (XSV), exhibited nucleotide and amino acid sequence similarity of less than 70% to representative soricomorph- and rodent-associated hantaviruses. Phylogenetic analysis, using maximum likelihood and Bayesian methods, showed that MOYV and XSV formed highly divergent lineages, distant from all other hantaviruses, except Magboi virus recently detected in the hairy slit-faced bat (Nycteris hispida) (family Nycteridae) from Sierra Leone. Suboptimal primer design and imperfect cycling conditions may have been responsible for the failure to detect hantavirus RNA in other insectivorous bat species.
Conclusions: The discovery of bat-borne hantaviruses heralds a new frontier in hantavirology. Many more hantaviruses are likely to be found in insectivorous bats throughout their vast geographic range. Intensive studies, including virus isolation attempts and high-throughput sequencing, are underway to clarify the phylogeography, ecology and pathogenic potential of these newfound hantaviruses.
Grant support: R01AI075057 and P20GM103516 from the National Institutes of Health.

Poster Session - Board 032 / 69

Interferon-gamma Production and Bactericidal Activity of Human Polymorphonuclear Leukocytes Exposed to S. aureus

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Polymorphonuclear leukocytes (PMNs) are phagocytic cells normally found in the blood stream able to migrate to sites of inflammation through chemotaxis. A previous study in our lab, using a mouse model of staphylococcal peritonitis, demonstrated that PMNs were the predominant source of interferon-gamma production which was induced by the SaeR/S two-component system in Staphylococcus aureus. Innate anti-bacterial mechanisms against S. aureus were diminished by interferon-gamma production in PMNs. In this current study, we investigated bactericidal activity of human PMNs exposed to conditioned serum against S. aureus. Furthermore, flow cytometry was used to analyze interferon-gamma production in human PMNs exposed to S. aureus and the induction effect of the SaeR/S two-component system. Our results suggest that conditioned plasma increases bacterial survival. Also, neutrophils appear to contain an intracellular pool of interferon-gamma. De novo interferon-gamma production by neutrophils exposed to S. aureus was observed by flow cytometry through antibody staining. These data suggest that a host blood component produced in response to S. aureus has deleterious effects on bacterial clearance. Human neutrophils appear to contain an intracellular interferon-gamma pool and de novo production of interferon-gamma occurs with exposure to S. aureus.

Breakout Session IIIB: Cell & Molecular Biology / 70

RecN stimulates RecA-mediated recombinational DNA repair of DNA double-strand breaks

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Background: DNA double-strand breaks (DSBs) are considered one of the most deleterious types of DNA lesions as their inaccurate repair leads to cell death or chromosomal instability. RecN, a protein critical to DSB repair in bacteria is highly expressed in response to DNA damaging agents and its function is critical to organisms known to tolerate high levels of oxidative DNA damage such as Deinococcus radiodurans and Neisseria gonorrhoeae. Bacterial RecN proteins share significant homology to the Structural Maintenance of Chromosomes (SMC) family of proteins. Eukaryotic SMC proteins have essential (although not fully understood) house keeping and tumor suppressor roles in a variety of DNA metabolic processes such as chromosomal condensation, sister chromatid cohesion and recombinational DNA repair.

Objective: Although extensive genetic evidence underscores the importance of RecN proteins to bacterial genome maintenance, there has been little substantive biochemical investigation into their function and, consequently, the specific reactions requiring RecN have not been identified. We previously determined that RecN is a cohesin-like protein that promotes intermolecular DNA interactions and hydrolyzes ATP. We set out to test if this DNA tethering activity stimulates key steps of recombinational DNA repair. Homologous recombination accurately repairs DSBs. The central bacterial recombinase protein, RecA, functions to mediate DSB repair via homologous DNA strand invasion to form D-loops. We tested the effect of the RecN protein on RecA-dependent activities in vitro.

Results: Here we present data demonstrating that 1) purified RecN protein greatly stimulates species-specific RecA-mediated D-loop formation acting at a step prior to DNA pairing; 2) RecA and RecN proteins interact; 3) DNA-dependent RecN ATPase kinetics are affected by RecA protein in a manner suggesting a specific order of protein-DNA assembly; and 4) Kinetic effects are independent of target homology but highly sensitive to target DNA concentration.

Conclusions: We present a model for RecN function that includes presynaptic stimulation of the bacterial DSB repair pathway and provide data that strongly suggests that RecN protein contributes to the search for homology. These novel findings open up new avenues of investigation in the bacterial recombinational DSB repair field and provides valuable models for understanding the molecular role that eukaryotic SMC proteins play in genome maintenance.

Poster Session - Board 089 / 71

Think Research: Chaminade's Undergraduate Pipeline

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Background: Chaminade University of Honolulu (CUH) holds a unique position as a Native Hawaiian serving institution that is assuming a position of education, research and community leadership in the Pacific. Since 2007 with multi-million dollar investment in facilities, faculty and instrumentation, CUH has transformed itself from a teaching college to a research enabled undergraduate institution.

Objective: Identifying CUH students who are interested in conducting research locally, nationally and even internationally.

Methods: Build the research into the curriculum at CUH and hire faculty who are committed to making research an integral component of a student's education.

Results: A pipeline of events have been outline for students interested in research from their freshman to senior year which consists of coursework, conference exploration, conducting research locally or nationally and presentation at undergraduate conferences. Every new hire in the STEM disciplines has come from an R1 institution with research interests that include environmental toxicology, reproductive health, immunology and obesity, cardiovascular disease, marine biology and biochemistry, forensic entomology and taphonomy, liver disease and cancer therapeutics. Our research laboratories are funded by NIH, NSF, DoD and foundation grants. Approximately 20 (some for multiple semesters) undergraduates participate in research internships on-campus annually. Every year, it is estimated that 20 (some for multiple years) undergraduates participate in Summer research internships locally or nationally. Besides providing partnership opportunities, these research internships culminate in student's attending, presenting and being recognized at conferences yearly.

Conclusion: CUH is committed to assisting and guiding any student who is interested in research opportunities as well as developing and maintaining functional and multi-faceted partnerships with leaders in education, research and community development.

Poster Session - Board 016 / 72

Using 3D cell culture to understand the mechanisms of fetal membrane rupture

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Background: Culturing cells in 3D is gathering momentum; under these conditions cells exhibit morphological and functional characteristics that recapitulate in vivo models. In addition to these advantages of studying cells in culture, it appears that re-building the tissue with 3D models may be the only way to study cells and their interactions within new extracellular matrix to understand the mechanisms of rupture of the fetal membranes. The two processes of ECM breakdown and apoptosis are central to the weakening of fetal membranes that occurs prior to membrane rupture at term but it is yet to be understood how these coalesce and how these may fail leading to preterm premature rupture of the membranes.

Objective: To develop and use novel 3D models to study cellular and extracellular matrix interaction to understand human fetal membrane rupture.

Methods: Human fetal membranes were collected at repeat Caesarian section at Kapiolani Hospital for Women and Children with prior IRB approval. Amniotic epithelial (AEC) and mesenchymal (AMC) cells were isolated using a method developed by Casey and McDonald (1). Cells were grown on plastic, in egg white albumin, hydrogel and alvetex matrices. Proliferating cell numbers were identified by BrDU and necrosing cells by propidium iodide incorporation. Immunocytochemistry was used to study; cellular morphology by phalloidin-TRITC labeling and NF-κB activation by p65 translocation. Pro-inflammatory stimulation of cells was performed by stimulation with either IL-1α or Pre-B cell colony-enhancing factor. Functional activation of NF-κB was confirmed by Luciferase construct activation and the downstream production of IL-8 and IL-6, as measured by ELISA.

Results: A comparison of AMC grown in plastic and in egg white albumin shows that in the latter the cells migrated, clustered and formed large networks over 7 days. Unlike 2D culture, these cells continued to survive for up to 40 days proliferating, (>95% viable). The cells also exhibited this morphology after growth in 2D culture (cells were passaged 5 times on plastic). Cell characteristics were then compared to 2D by the growth of both AEC and AMC also in Alvetex scaffolds and Hydrogel. Cells readily adhered to both 3D media were imaged and modeled three-dimensionally so that their spatial relationships and interactions with the environment studied. As inflammation drives ECM remodeling, the activation of NF-κB upon stimulation with various pro-inflammatory cytokines was confirmed. Changes in the levels of apoptosis by cytokine stimulation was also detected in these cells.

Conclusion: This data confirms previous reports that these cells have the potential to drastically respond to their environment, and that inflammation and apoptosis can be modeled so that we can study their interaction in this tissue to understand the mechanisms of fetal membrane rupture.

Poster Session - Board 034 / 73

Formation of Dynamic Membrane Structures in Activating Mast Cells

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Background: Mast cells represent a remarkable system in which to study the dynamic membrane changes that accompany antigen receptor activation. The signaling events and functional responses that follow antigen receptor engagement in mast cells are critical to inflammation. Massive membrane rearrangement, in the form of acquired phospholipid asymmetry, exosome formation, vesicularization and degranulation of secretory granules, follows antigenic ligation of the high affinity receptor for IgE, FcƐRI. With no loss of membrane integrity, mast cells manifest these changes during activation and then recover, and are able to complete numerous activation-recovery cycles throughout their lifetimes.

Objective: The interest of the PI's research program is to study membrane dynamics and the role of non-apoptotic phosphatidylserine flipping during vesicle formation.

Methods: To characterize the membrane dynamics during vesicle formation in the mast cell, we will be using sophisticated imaging, biophysical and cell biological techniques. Recent technical and intellectual advances create the opportunity to solve some of the outstanding, yet fundamental, questions as to the role of the membrane's composition and behavior in control of mast cell activation, and to identify novel control mechanisms that may be targeted for the tuning and modulation of mast cell-driven pro-inflammatory responses.

Results: Our preliminary data establish a model system for the study of altered lipid asymmetry, exosome formation, and vesicularization following FcƐRI activation of mast cells.

Conclusion: The use of this model system allows us to study a possible role for the controlled, non-apoptotic, phosphatidylserine (PS) re-distribution between the cytoplasmic and extracellular leaflets of the plasma membrane is a common requisite for the membrane proximal signaling events, the dynamic formation of exosomes and degranulation, and mast cell functional responses that follow FcƐRI ligation.

Poster Session - Board 090 / 74

I Am A Scientist

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Background: The I am a Scientist STEM outreach program at Chaminade University of Honolulu inspires and excites students by engaging their curiosity and provides them with the scientific tools to discover their own answers to life's problems.

Objective: Recognizing the pressing need in Hawaii and across the nation to engage and guide young students into the STEM pipeline, the I am a Scientist program was specifically designed to: (1) Intervene early: focus outreach programs on elementary schools; (2) Engage and educate: go beyond purely enrichment activities to actively partner with under-resourced schools to help them deliver curriculum and meet standards; (3) Sustain contact: repeated visit so that students maintain constant awareness of the excitement of science and encounter role model scientists and undergraduates repeatedly; and (4)
Cultural awareness: communicate the relevance and importance of science in a manner that is meaningful to all students.

Methods: Our program is unique in that we work closely with the teachers to customize modules to complement their curriculum and work within the school's bell schedule. This enables our program to support the teachers, and address the Hawaii Content and Performance Standards (HCPS) in Science as set by the Hawaii Department of Education (DOE). Furthermore, by going to the schools, we eliminate transportation cost and travel time for the students. We also offer evening assembly style programs so families can learn together. The program is offered at no charge, and each participating student and teacher receives their very own white lab coat.

Results: Collaborating with the Chaminade University faculty, staff and students, we are able to provide an exceptional science experience to our youth. Student service club members provide instruction and serve as role models for the participants. An added benefit to our Chaminade students is the experience of teaching science, and the reinforcement of what they learned in school.

Conclusions: Our faculty and staff, puts a face to what a professional scientists is, and are able to encourage the youth and families to be excited about school and STEM subjects. Our program modules include Health Science, Biochemistry, Genetics, Chemistry, and Forensic Science.

Poster Session - Board 015 / 75

Human Amnion Epithelial and Mesenchymal Cells Express Telomerase

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Background: Although cells isolated from the fetal membranes are studied in parturition research, many of their basic characteristics remain unknown. These cells are derived from the fetus early in pregnancy and may have pluripotent capacity.

Objective: The purpose of this study was to compare telomerase expression in cells isolated from human fetal membranes with cancer cell lines. Our lab routinely isolates both epithelial cells and mesenchymal cells from the human fetal membranes to study the mechanisms of parturition. However, these cells often exhibit differential proliferation and growth characteristics. Both stem cells and cancer cells have high levels of telomerase, which correlates with their ability to rapidly proliferate. Therefore we chose to determine if the telomerase expression could provide a functional marker for their proliferative capacity.

Methods: In order to compare how different cancerous cell lines (HEK, WISH, A549) and primary cells proliferate in culture we seeded them at 750,000 and 1 million cells per well on a 6 well plate (cell lines and primary cells respectively). On subsequent days, the cells were trypsinized and counted. Growth/proliferation was also monitored by MTT assay. Lastly, real-time PCR was performed on the cell lysates to measure the expression of telomerase by two methods (QTD kit; Allied Biotech Inc. and Trapeze RT; Chemicon).

Results: The cell counting proliferation data demonstrated that all three of the cancerous cell lines proliferated twice as much and their peak growth rates were on average 5 fold higher than the primary cells. The MTT data did not reflect the proliferation assay results. Both telomerase real-time PCR assay demonstrated that all of the cells expressed telomerase. They also demonstrated that the cancer cell lines had more telomerase expression than our primary cells of interest. While there has been controversy in the literature as to whether human fetal cells express telomerase, we show clear expression by two methods.

Conclusion: This data supports the idea that telomerase expression could be used to determine the proliferative capability of our cells. Our data also supports the mounting evidence by other researchers that these cells may be a source of therapeutic stem cells.

Poster Session - Board 029 / 76

The Use of MALDI Imaging to Validate Tissue Markers of Pressure Overload-Induced Cardiac Hypertrophy and Heart Failure in Mice

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Background: Cardiac hypertrophy is an adaptive response of the heart to many forms cardiac disease. While the hypertrophic response is initially a compensatory mechanism that augments cardiac output, sustained hypertrophy can lead to dilated cardiomyopathy and heart failure. Heart failure is a major cause of morbidity and mortality worldwide and is a health disparity in Native Hawaiian and Pacific Islander populations. Matrix-assisted laser desorption/ ionization imaging, or MALDI imaging, is a novel technique that reveals how peptides are spatially distributed within a given sample by measuring mass spectra of intact tissue sections.

Objective: The objective of this study was to compare the use of MALDI imaging with traditional methods for detecting known tissue markers or murine cardiomyopathy.

Methods: Wild type C57BL/6J mice were subjected to transverse aortic constriction (TAC) or sham surgery for 2 weeks. Hearts were retrieved and prepared for RNA, protein and MALDI histological analyses.

Results: TAC-treated mice showed increased mass spectral abundance of hypertrophic biomarkers of fibrosis, tissue remodeling and inflammation compared to sham mice, and was confirmed by gene and protein expression analyses.

Conclusion: These results suggest that MALDI imaging is a useful tool in assessing spatial protein changes in modeled cardiac hypertrophy and heart failure in mice.

Poster Session - Board 005 / 77

Neuroprotective action of a N-terminal fragment of beta amyloid

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Background: Beta amyloid (Aβ) is the primary component of the neuritic plaques associated with Alzheimer's disease (AD). However, Aβ is present at relatively low levels (picomolar) in normal brain, where it appears to have a neuromodulatory role. In particular, we have demonstrated that pM Aβ has an agonist-like action at synaptic nicotinic acetylcholine receptors (nAChRs). In contrast, prolonged exposure to elevated levels (nM-uM) of Aβ was shown to have toxic effects on neuronal systems, especially in the presence of nAChRs. In an attempt to characterize the essential structural elements in Aβ, we discovered that a N-terminal fragment, Aβ1-15, accounts for the agonist-like activity of full-length Aβ, but is not toxic. In fact, Aβ1-15 was shown to be more active and much more potent than full-length Aβ in functional studies. Aβ1-15 is naturally the product of the cleavage of Aβ by α-secretase.
Objective: To examine possible neuroprotective effects of the non-toxic N-terminal fragment Aβ1-15 by examining the levels of reactive oxygen species (ROS), nuclear integrity and cell viability of neuroblastoma cells simultaneously treated with toxic levels of full-length Aβ.
Methods: Differentiated NG108-15 rodent hybrid neuroblastoma cells were used as model neuronal system. Mouse sequences for α4- and β2-nAChR subunits housed in expression vectors were transiently transfected into the differentiated neuroblastoma cells. Mock-transfected NG108-15 cells were used as controls. Treatment conditions included: Untreated; Treated with 100nM Aβ1-42 (full-length) alone; Treated with 100nM Aβ1-15 alone; or Co-treatment with 100nM Aβ1-15 and 100nM Aβ1-42. The Image-iT Live Reactive Oxygen Species (ROS) detection was used to quantify oxidative stress and nuclear integrity. Cell viability was assessed via standard cell counts.
Results: The results showed that 100nM of the N-terminal fragment Aβ1-15 blocked the increase in ROS in differentiated neuroblastoma cells expressing nAChRs following treatment with Aβ1-42 for 3 days. Aβ1-15 also blocked disintegration of nuclei following 3-day treatment with Aβ1-42. Consistent with previous results, Aβ1-15 alone was without effect, confirming that it is non-toxic. Interestingly, Aβ1-15 also blocked the more modest increase in ROS induced by Aβ1-42 in mock-transfected neuroblastoma cells, which are devoid of nAChRs.
Conclusion: The neuroprotective action of the N-terminal beta amyloid fragment Aβ1-15 against the neurotoxicity induced by elevated levels of full-length Aβ indicate that the N-terminal Aβ fragment may serve a competitive compensatory function at the synapse in the context of accumulating Aβ. Moreover, our results suggest a promising new possibility for AD therapy.

Poster Session - Board 076 / 78

SirX: a fast, memory-efficient reference based de novo transcriptome assembly pipeline

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Transcriptomic assembly of high-throughput RNA-seq datasets, often consisting of terabases, poses a significant computational challenge, particularly in terms of memory requirements.

In this project we describe SirX, a fast and memory-efficient RNA-Seq assembly pipeline that uses protein references to reconstruct a snapshot of the organism's transcriptome, in the absence of a reference genome.

In the SirX pipeline, we apply a divide-and-conquer strategy to partition the large-scale assembly problem into numerous smaller instances. In using a reference-based approach, large high-throughput datasets can be partitioned into isotigs and processed independently and in parallel. This process results in substantial improvements in memory requirements compared to current transcriptome assembly applications, which often require de-replication or normalization to process the billions of RNA-seq reads produced by second-generation sequencers.

The SirX pipeline comprises 3 stages: in the first stage, RNA-seq reads are aligned to protein sequences using a fast alignment heuristic. The resulting alignments partition the initial dataset into many smaller, possibly overlapping subsets, each representing an isoform. In order to minimize the likelihood of erroneous partitions, various heuristics are applied, and reads with low-confidence assignments are removed. Scaffolds of the resulting partitions are subsequently derived without explicitly resorting to assembly methods, thus yielding faithful, short consensus sequences of protein coding regions of the studied organism. In the second stage, the short contigs are further extended, using a k-mer based approach. Finally, the reads from novel proteins, i.e., those that do not align against any known reference, are processed independently using traditional layout-overlap-consensus or de Bruijn assembly methods.

Our results show that using SirX, the fraction of assembled transcriptome depends upon the availability of proteins with sufficient sequence similarity to those of the studied organism. Nevertheless, even in the absence of references from a taxonomically close organism, our approach can reconstruct a substantial fraction of the transcripts' catalogue, therefore significantly reducing the complexity of the dataset that needs to be assembled using existing methods.

Breakout Session IIIC: Health Disparities / 80

Evolution of a Community-Based Participatory Research COBRE at the University of Alaska Fairbanks

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Background: The Center for Alaska Native Health Research (CANHR), originally focused on genetic, nutritional and behavioral risk and protective factors for obesity and related co-morbidities, has expanded to include the health priorities of Alaska Native people that include intervention research on the prevention of obesity, cancer, suicide and substance abuse.

Objective: Successful community engagement in all phases of the research process has increased community members trust of, and involvement in, biomedical and behavioral health research. However, community requests are often outside the expertise of CANHR investigators.

Methods: To address the diverse health priorities of Alaska Native people, we are expanding our scientific networks by collaborating with research scientists that have expertise in a variety of disciplines that are not well represented at our university. In our COBRE I award, we developed a university President's Professor program to provide mentorship and stimulate collaborative opportunities with scientific leaders in areas congruent with CANHR faculty interests. In addition, we initiated a Pilot grant program in our COBRE III award that requires early stage investigators to expand their collaborative networks through engagement of external scientific leaders that have specific expertise relevant to the proposed pilot projects and a willingness to mentor our investigators.

Results: Several collaborative proposals have been funded and the collaborative networks of CANHR are expanding. Examples of how our collaborative networks have grown in intervention research, genetic ethics, pharmacogenetics, nutrition and behavioral sciences research will be presented.

Conclusions: We illustrate one model of how the IDeA program has catalyzed the path to sustainability of a COBRE-funded center that has built a respectful trusting research partnership with Alaska Native people and addresses health concerns of Alaska Native communities through the formation of collaborative networks. We invite additional collaborations in Alaska Native priority research areas from COBRE and INBRE centers throughout the western region.

Breakout Session IIIC: Health Disparities / 81

Understanding and Addressing Disparities in Safe Drinking Water Access on the Crow Reservation

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Background. Nationwide, about 12% of American Indian/Alaskan Native homes lack access to safe drinking water and/or basic sanitation (IHS 2010 data). However, our understanding of "disparities associated with water infrastructure" is very limited (VanderSlice 2011, AJPH).
Objectives. The Crow Environmental Health Steering Committee (CEHSC), a coalition of Tribal stakeholders and academic partners, has been working together since 2006 to assess risks from contaminants in domestic, cultural and recreational water sources; improve access to safe drinking water and sanitation; and increase community capacity to address water quality issues on the Crow Reservation, Montana.
Methods. Local river, spring and home well water samples have been collected and analyzed for microbial and chemical contaminants. Well owners completed surveys about their water sources and uses, and have received written reports explaining test results and water treatment options. A low cost, high tech home water filtration system was pilot tested. In-home visits and project evaluation with participants are underway. Interviews with 30 key informants were conducted to better understand the complex impacts of local water contamination. GIS and MS Access are being used for data entry and analysis. Public education efforts, including teacher training, are ongoing. Our project follows community-engaged research (CER) principles, with the CEHSC guiding the work and being involved in nearly all phases including publication.
Results. 55% of home wells tested present health risks to users from chemical and/or microbial contamination. Risk factors for coliform contamination of home wells have been identified. Most well owners prefer in-home visits by project staff to explain test results and treatment options. High levels of fecal contamination in rivers make them unsafe for ongoing cultural uses, periodically unsafe for swimming, and a high risk source for municipal water treatment. Adults who grew up playing along the rivers are (reluctantly) restricting their children's recreational uses of the rivers due to contamination, but most maintain vital cultural uses of river water despite health risks. The data obtained have supported successful Apsaalooke Water and Wastewater Authority grants totaling more than $20 million to date for repairs and upgrades to Crow Agency's water and wastewater systems. 20+ Tribal college science majors have gained research experience as project interns. Many legal, regulatory, jurisdictional and economic factors contribute to water quality disparities in Reservation communities.
Conclusion. Understanding the complex causes and impacts of water contamination in Reservation communities requires CER, a multidisciplinary analysis utilizing mixed methods, and the flexibility to follow research leads as they unfold. Mitigation for municipal water has been largely successful. Better solutions are needed for families maintaining cultural uses of the rivers and for well owners, as financial constraints limit implementation of existing well water treatment technologies.

Poster Session - Board 066 / 82

Determining Coliform and E. coli Levels in Pryor Creek

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Water quality is of great concern to the Crow Tribe. Surface waters such as Pryor Creek near Pryor, MT on the Crow Indian Reservation in Southeast Montana.are used for many purposes, including drinking (both for people and livestock), recreation, and traditional purposes. This particular study is designed to provide feedback to the Crow Tribe on the quality of water in Pryor Creek, focusing on the extent of fecal contamination. The local community is concerned about whether there may be contamination of the creek due to its close proximity to a primary treatment wastewater lagoon and an old and potentially leaking waste water pipe that crosses over the creek to empty into the lagoon. In addition, livestock have direct access to the creek which introduces fecal matter into the creek. Contamination of the creek is not only a concern for those who directly use the water but also could have an impact on shallow drinking water wells. We hypothesize that both coliform and E. coli bacteria will be present in the creek and levels of these bacteria are being assessed.

Water samples are collected from thirteen sites along Pryor Creek, including near the lagoon, upstream and downstream of the lagoon, near the waste water pipe, and directly from two sacred springs. Samples are collected in sterile 500-mL glass bottles from the middle of the creek, or in areas with higher flow rates. Samples are kept on ice until return to the RMC laboratory, where the IDEXX Colilert water testing system is used to quantify coliform and E. coli numbers using a Most Probable Number approach. Samples are run undiluted and at 2-fold, 5-fold, and 10-fold dilutions as appropriate.

Both coliform bacteria and E. coli have been observed in the water samples. The bacterial levels vary from site to site and over time and trends are being examined. The sacred springs have very low levels while levels near sites with higher cattle use can be high. There does not appear to be any contamination of the creek due to the lagoon or wastewater pipe to date. This study is ongoing, and final conclusions will be made after the completion of the study in April 2014.

Poster Session - Board 054 / 83

Lipid body dynamics in live mast cells

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Background: We have previously shown that insulin exposure in mast cells (MC) is associated with pronounced lipid body formation, suggesting that immunocytes, like hepatocytes and myocytes, can be sites for ectopic lipid accumulation when insulin levels are systemically dysregulated. Lipid body accumulation in mast cells (MC) is accompanied by suppressed secretory response and cytokine gene induction, but enhanced release of arachidonate-derived bioactive lipids (e.g. leukotriene C4). Macro-cytochemical staining suggests that LB are dispersed after mast cell activation, but the fate of the LB has not been tracked at the subcellular level or in real time.

Objective: In this study we tested the hypothesis that lipid bodies will be spatially relocated after stimulation is applied to mast cells (RBL2H3).

Methods: After being stained with Oil Red-O (ORO) as the identifying marker for lipid bodies, we mimicked antigen receptor ligation using Phorbol 12-Myristate 13- Acetate (PMA) and ionomycin to simulate single live mast cells. Confocal and epi-fluorescent imaging were performed on a Nikon Ti Eclipse fluorescence microscopy system in 2 and 3 dimensions and over a time course of 15 min. Lipid body mobilization was tracked and analyzed in NIS Elements (Nikon). Movement was observed when comparing resting mast cells with PMA/ionomycin treated cells.

Results: We observed changes in the relative position, size, and morphology of lipid bodies, apparent dispersal of lipid body contents and both LB fusion and apparent formation of new lipid bodies.

Conclusions: Thus lipid bodies in mast cells are highly dynamic, and this study serves as a foundation to evaluate the links between mobilization of pro-inflammatory bioactive lipids in activated mast cells and the microscopic behavior of the lipid bodies themselves.

Breakout Session IA: Neuroscience / 84

Selenoprotein P in Synaptic Physiology and Neurodegenerative Disorders

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Background: Selenoprotein P (Sepp1) is an extracellular protein with multiple selenocysteine residues involved in transport of selenium. Association of Sepp1 with pathology in neurodegenerative disorders as well as neurological impairments in Sepp1 knockout (Sepp1KO) animals demonstrates the importance of Sepp1 for brain function. However, the direct actions of Sepp1 in the brain and the mechanisms of selenium delivery are poorly understood.
Objectives: The objectives of our studies were to determine the relationship of Sepp1 expression to pathology in Alzheimer's and Parkinson's postmortem brain, as well as a possible role for Sepp1 in synaptic physiology.
Methods: We have investigated the neural expression of Sepp1 with immunohistochemistry in human postmortem brains with Alzheimer's and Parkinson's disease. We also examined the role of Sepp1 in synaptic physiology using hippocampal slices prepared from wild-type and Sepp1KO mice. Additionally, we investigated restoration of Sepp1 specifically in the brain of Sepp1KO mice.
Results: We found Sepp1 to be associated with both Alzheimer's and Parkinson's brain pathologic lesions. Deletion of Sepp1 results in bidirectional changes in synaptic plasticity. Deficits observed in Sepp1KOs in long-term potentiation (LTP), a cellular model for learning and memory can be reversed by introducing Sepp1 directly in the hippocampus, indicating a direct action of Sepp1 on neuron physiology.
Conclusion: These studies demonstrate the importance of Sepp1 in maintaining synaptic integrity and plasticity.

Poster Session - Board 063 / 85

Investigation of Infrared Vibrational Frequency as a Molecular Descriptor for the Lead Optimization of PKB/Akt Inhibitors

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Background: The lead optimization to discover a drug candidate in the pharmaceutical industry is a rate-limiting and costly process; primarily guided by the lock and key theory. This method of approach for ligand to receptor binding optimization lacks clarity in determining the design of the best ligand or inhibitor.

Objective: We hypothesize the vibrational bond frequencies of ligand is an important and novel factor for binding interaction with the protein target.

Methods: We applied this method for the lead optimization of two chemical series: pyridinopyrazines and quinoxalines at one of the most elusive oncology targets, Protein Kinase B (PKB). Compounds in these two series were designed based on the summation of the theoretical infrared absorptions at each bond normalized by their molecular weight (MDIR value). Twenty-eight compounds were then synthesized via Suzuki Coupling or Reductive Amination. Purity assessment was done via LCMS, FTIR, and 1H-NMR. The PKB inhibitory potency of each compound was evaluated using an ELISA based assay. IC_{50} was evaluated for all compounds with PKB inhibition >20% at 10μM. Correlation between the MDIR or FTIR values and IC_{50}s were assessed for lead optimization.

Results: A positive parabolic relation between MDIR and PKB inhibitors was observed in quinoxaline analogs with an IC_{50} range of 1.8 – 11.3 μM.

Conclusion: The use of MDIR provides a novel modality for lead optimization in drug discovery.

Breakout Session IA: Neuroscience / 86

Striatal neuronal loss occurs in the R6/2 mouse model of Huntington's disease.

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Background: Human Huntington's disease (HD) is a chronic neurodegenerative disorder that results in brain striatal neuronal degeneration and loss leading to atrophy and disease manifestations. The R6/2 mouse model of HD demonstrates many features of human HD; however, it is unknown whether striatal neuronal loss occurs. Determining the presence or absence of striatal neuronal loss in R6/2 mice is important as it will influence choice of mouse HD models for studying therapies designed to rescue neuronal loss.

Objective: Determine if neuronal loss occurs in R6/2 HD mouse striatum.

Methods: Striatal neuronal numbers were estimated using an un-biased stereologic approach. Analyses were completed in R6/2 HD and wild-type litter-mate mice at 5-weeks (pre-clinical) and 12-weeks (late disease) of age. Mouse pups were genotyped at three weeks of age by PCR on gDNA derived from tail tips. At 5 and 12-weeks mice were anesthetized then perfused through the left ventricle with 4% paraformaldehyde fixative. Brains were sectioned at 40 μm, then every 12th section was mounted onto a glass slide and stained using the thionin method. Striatal neuronal estimates were made using the optical fractionator technique. Data was analyzed using ANOVA in SAS software. Results are presented as means ± standard errors.

Results: At 12-weeks R6/2 striatum had 12.7 % fewer neurons than wild-type litter mates (868043 ± 38483 and 993890 ± 38483, respectively: p=0.0266). At 5-weeks there was no difference in striatal neuronal estimates between R6/2 HD and wild-type mice (1003945 ± 45789 and 1061500 ± 42124, respectively: p=0.3688).

Conclusion: The findings indicate that R6/2 HD mice have significantly fewer striatal neurons at 12-weeks of age, corresponding to late-stage disease. As this could be interpreted as a result of neuronal loss or possibly a developmental anomaly we decided to undertake the same analysis in 5-week-old mice. The lack of significant findings at this age indicates that R6/2 HD mice lose neurons after 5-weeks of age. By 12-weeks of age their striatum have ~127000 fewer neurons than wild-type mice. Findings indicate that R6/2 mice are valuable for studying mechanisms of neuronal loss and therapies aimed at decreasing neuronal loss.

Breakout Session IIIC: Health Disparities / 87

Gender Differences in the Prevalence of Obesity and Associated Cardiometabolic Factors among Western Alaska Native People: The Western Alaska Tribal Collaborative for Health (WATCH) Study

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Background: Obesity is associated with increased risks of cardiovascular disease, type 2 diabetes, and other chronic diseases. Obesity risk varies among different ethnic groups. Prevalence estimates for metabolic disorders are well documented in European and certain ethnic populations, but Alaska Native groups are understudied. The Western Alaska Tribal Collaborative for Health (WATCH) Study combines data from three western Alaska Native cohorts.
Objective: To assess the prevalence of overweight and obesity, and cardiometabolic risk factors, and the relationship between obesity and these risk factors, by sex in the WATCH study population.
Methods: Analyses were based upon a sample of 3985 Yup'ik and Inupiat participants, 2140 women and 1845 men, ≥18 yrs. with a mean age of 39.9 yrs. Anthropometric measurements were categorized using clinically relevant cut-points. The prevalence of obesity and associated metabolic risk factors for cardiovascular disease and type 2 diabetes were assessed by gender according to NCEP/ATP III guidelines. Regression analysis was used to assess the association between obesity and cardiometabolic risk factors, including lipids, blood pressure and glucose.
Results: Overall mean BMI for women was higher than for men (29.1 vs. 26.5, respectively).The prevalence of obesity (BMI ≥ 30) was higher in women (40%), than men (20%), Only 18.6% of men had a waist circumference (WC) >102cm, while 58% of women had a WC >88cm ($p<0.001$). Women had higher HDL-C, and triglyceride levels compared to men, while systolic blood pressure (SBP), diastolic blood pressure (DBP), LDL-C, and glucose were higher in men than in women. In multivariate analyses, BMI and WC were significantly associated with all of the cardiometabolic risk factors. The association between BMI and DBP, HDL-C, and triglycerides was significantly stronger for men than women. Similarly, the association between WC and both LDL-C and triglycerides was significantly stronger in men than women. In all of the multivariate analyses, only the association between WC and SBP had a negative interaction effect for men.
Conclusion: The high prevalence of obesity and central adiposity in Alaska Native women is a serious health concern. Similar to other populations, these levels are correlated with increases in cardiometabolic risk factors among both men and women. The multivariate analyses, however, suggests that the higher rates of obesity in women may have less adverse effects than expected. Future research is needed to investigate gender-specific cardiometabolic factors and obesity, with the goal of developing culturally relevant and effective interventions to reduce cardiometabolic disease risk.

Breakout Session IA: Neuroscience / 88

Psychoactive Pharmaceuticals Induce Fish Gene Expression Profiles Associated with Human Idiopathic Autism

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Idiopathic autism, caused by genetic susceptibility interacting with unknown environmental triggers, has increased dramatically in the past 25 years. Identifying environmental triggers has been difficult due to poorly understood pathophysiology and subjective definitions of autism. The use of antidepressants by pregnant women has been associated with autism. These and other unmetabolized psychoactive pharmaceuticals (UPPs) have also been found in drinking water from surface sources, providing another possible exposure route and raising questions about human health consequences. Here, we examined gene expression patterns of fathead minnows treated with a mixture of three psychoactive pharmaceuticals (fluoxetine, venlafaxine & carbamazepine) in dosages intended to be similar to the highest observed conservative estimates of environmental concentrations. We conducted microarray experiments examining brain tissue of fish exposed to individual pharmaceuticals and a mixture of all three. We used gene-class analysis to test for enrichment of gene sets involved with ten human neurological disorders. Only sets associated with idiopathic autism were unambiguously enriched. We found that UPPs induce autism-like gene expression patterns in fish. Our findings suggest a new potential trigger for idiopathic autism in genetically susceptible individuals involving an overlooked source of environmental contamination.

Poster Session - Board 072 / 89

LINE-1 hypomethylation and radiation-induced genomic instability in industrial radiographers

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Background: Genomic instability is an important factor in cancer induced by ionizing radiation. Global DNA hypomethylation has been recently proposed as a potential biomarker for cancer risk through genomic instability. However the association between low-dose ionizing radiation exposure and DNA methylation changes is unclear.
Objective: The aims of this study were (1) to identify the association between low-level radiation exposure and genomic hypomethylation, and (2) to determine the relationship between genomic hypomehtylation and radiation-induced genomic instability in industrial radiographers.
Methods: Genomic hypomethylation of repetitive element LINE-1 was measured in WBC DNA from 40 industrial radiographers and 32 healthy male volunteers using pyrosequencing assay. The micronucleus-centromere assay was also performed to measure aneuploidy of chromosome 1 and 4.
Results: The mean level of LINE-1 methylation was significantly lower in radiographers than in controls (73.8 ± 1.2% vs 74.4 ± 1.1%, $p<0.05$). LINE-1 hypomethylation was not significantly correlated with the last recent 1-year, the last recent 5-year, or the total cumulative radiation doses in radiographers. However, LINE-1 hypomethylation was significantly correlated with the cumulative radiation dose without recent 5-year exposure ($r = -0.38$, $p<0.05$). Also, LINE-1 hypomethylation was a significant contributor to total aneuploidy by the cumulative radiation dose without recent 5-year exposure (37% to 44% variation of total aneuploidy, $p<0.05$).
Conclusion: Our data indicate that LINE-1 hypomethylation is associated with the delayed genomic instability induced by radiation, but additional studies with a larger number of subjects and other repetitive elements with different assays are needed to fully understand the relationship between genomic DNA hypomethylation and radiation-induced genomic instability.

Poster Session - Board 070 / 90

Nanotoxicity: An interdisciplinary problem

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Over the last two decades, incorporation of nanomaterials in consumer products, high-tech devices, and research projects have increased exponentially. Thus, it is likely that the exposure from the presence of nanomaterials will increase. There have been a number of reports dealing with nanomaterials and their potential environmental and health effects. However, due to the inherent difficulties of characterizing nanomaterials and the fact that the field of nanoscience is in constant flux with new questions, materials, and applications, trying to understand nanotoxicity in is a time-consuming, complex and difficult problem. Thus far, many studies have focused on nanomaterials that are commercially available and easier to prepare. In our studies, however, we are interested in technologically important nanomaterials such as CuInS2 nanoparticles, which has been prepared to be use in photovoltaics and optical sensors. Through a close collaboration between chemists and biologists, first, an INBRE summer student and mentors prepared and characterized several batches TiO2 and CuInS2 nanoparticles and, second, evaluated their effects on pulmonary cell. We have examined the effects based on sizes, phases, and synthetic methods of nanoparticles. We employed several biological assays, such as cell titer blue assay, flow cytometry, and reactive oxygen species measurements. We found that anatase phase and rutile phase of TiO2 appears to have significant differences in measurement of reactive oxygen species. Surprisingly, CuInS2 nanoparticles, a small bandgap semiconductor, had most profound effect in measurement of reactive oxygen species thus far. We also found significant differences in particle uptake by cells between different phases of TiO2 nanoparticles.

Poster Session - Board 058 / 91

Physiological function of the TMC protein family

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Background: Mutations in either of two adjacent genes located on 17q25: Tmc6 (Ever 1) or Tmc8 (Ever 2) are causative in the disease Epidermodysplasia verruciformis (EV). This rare autosomal recessive genodermatosis results from an abnormal susceptibility to human papillomavirus (HPV) genotypes and to their oncogenic potential. Infection with HPV (mainly HPV5) leads to the early development of disseminated flat wart-like and pityriasis versicolor-like lesions akin to verrucae planae, which patients cannot reject. Cutaneous Bowen carcinomas in situ and invasive squamous cell carcinomas develop, usually in areas exposed to sun.

Objective: The central goal of this project is to establish the mechanism by which this disease progresses, and reveal the enigmatic physiological functions of the TMC6/8 proteins.

Methods: pcDNA5/TO vector containing tetracycyline-inducible FLAG- or 6xHIS-tagged human TMC cDNAs were cloned into HEK293TRex cells to generate stable cell lines, and transiently transfected with V5-PRDX2 or V5-NME1 and co-immunoprecipitated with TMC8 to show protein interaction. Overexpressing cell lines, normal primary keratinocytes and human skin sections were assessed for TMC8 protein expression using western blot or immunohistochemical analyses and compared to tumor tissue lysates or squamous cell carcinoma human skin tissue arrays.

Results: The data presented here documents two areas of progress. First our data indicate interaction of TMC8 with NME1, a component of the SET complex, and PRDX2, a component of the ROS signaling and regulatory pathways. In vitro and biochemical confirmation of these interactions are ongoing. Second, we are in the final stages of developing two novel knockout mouse strains: a systemic tamoxifen-inducible TMC8 knockout and a tamoxifen-inducible keratinocyte-specific TMC8 knockout.

Conclusions: We are therefore close to having in hand an unprecedentedly powerful set of in vivo tools for the study of TMC proteins and EV. At the end of this project, we will have identified new and critical mechanisms in viral surveillance and or oncogenic progression.

Breakout Session IIIB: Cell & Molecular Biology / 92

Evolution of G-matrix of stress tolerance in yeast

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Understanding the evolution of complex traits such as stress tolerance has long been a challenge for evolutionary biologists. While quantitative genetics offers a framework to study the patterns of variation as seen by selection, there has been no attempt to link these to the underlying gene metabolic networks and their effects on trait evolution. We use salt- and glycerol- stress tolerance in yeast (Saccharomyces cerevisiae) to study how the gene networks involved generate trait variation and covariation at the quantitative genetic level. Two divergent strains of yeast (DBVPG1106 and YPS128) were crossed to generate an admixed population. Growth rates were measured in four different stressing environments with varying concentrations of salt and glycerol (0-2% and 0-8% respectively). We measured G-matrices of quantitative genetics to estimate (co)variation between these four fitness components among multiple genotypes in different generations. We found differences in stress tolerance between the two parental strains as well as within the admixed population. This suggests that a trade-off exists in how the cell can cope with different levels of stress. The two underlying metabolic networks are tightly linked through gene-gene interactions and the genetic variance and covariance structure resulting can impose an evolutionary constraint during adaptation to stress. Further manipulative evolution experiments will enable us to track how selection shapes G as a function of the gene-gene interactions motifs.

Poster Session - Board 062 / 93

Structural-Functional Studies of TSPO

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The 18-kDA human translocator protein, TSPO, is an important drug target in a variety of human diseases, including heart disease and diabetic neuropathy. TSPO is part of the mitochondrial transition pore, and it has an important role in regulating mitochondrial functions. There is no high-resolution structure available of the human TSPO or any of its homologs, which limits our understanding of the molecular mechanism of TSPO in health and disease. As TSPO is widely distributed and has multiple functions, it is very important to identify the structural functional significance of ligands before their efficient application to treat specific diseases. The long-term goal of this project is the characterization of the multiple ligand binding sites and the high-resolution structure determination of TSPO by Nuclear Magnetic Resonance (NMR) spectroscopy and other biophysical methods. We cloned a well behaved structural and functional homolog of TSPO from Rhodobacter sphaeroides into an E. coli pET23a vector, and we optimized the expression and purification. For solid-state NMR studies, detergent solubilized TSPO was reconstituted into E. coli polar lipids. NMR chemical shift analysis confirmed that the protein was successfully reconstituted into the lipids in its native α-helical conformation, which is further supported by secondary structure analysis by circular dichroism. Intrinsic tryptophan fluorescence quenching experiments indicated that TSPO was expressed and purified in a functional state. Results of this study confirm that TSPO is suitable for solid-state NMR structure determination, and further experiments are under way. A high-resolution structure of TSPO is expected to be transformative for drug design to combat heart disease, diabetic neuropathy, and other disorders.

Poster Session - Board 073 / 94

The Partial Genome Structure of Star Jasmine Mosaic Virus in Hawaii

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The leaves of some star jasmine plants in Hawaii display a mosaic symptom typical of virus infection. A few virus diseases have been well characterized in different jasmine species but the symptoms associated with these diseases are distinct from those observed in Hawaii. The objective of this research is to identify and molecularly characterize any viral pathogens associated with the disease symptoms observed in the Hawaii star jasmine plants.

Symptomatic star jasmine leaves were collected from Manoa Valley, Oahu. DsRNA was extracted from 10 g of leaf samples and DsRNAs were analyzed by electrophoresis. The dsRNAs were converted to complementary DNA followed by digestion and then overlap-extension (OE) PCR was performed to produce dsDNA. The resulting PCR fragments were ligated into pGEM-T Easy (Promega) and recombinant plasmids were sequenced at the University of Hawaii's Advanced Studies in Genomics, Proteomics, and Bioinformatics laboratory. Sequences were assembled using CAP3 program and resulting contigs and singlets were identified using BLASTX program. The RdRp portion of the virus genome was aligned with other related viruses using ClustalW and a phylogenetic tree was constructed using a neighbor-joining algorithm. Double-stranded RNAs approximately 1059 and 1383kb in size were isolated from symptomatic star jasmine plants. Sequence data indicated the presence of at least two distinct viruses, both of the Family Tombusviridae. Phylogenetic analysis using the RdRp indicated one of these viruses is most closely related to Pelargonium line pattern virus, and the other to Beet black scorch virus. We tentatively propose the names Jasmine mosaic-associated virus 1 (JaMaV-1) and JaMaV-2 for these viruses. Further molecular characterization of the viral genomes and pathogenicity trials to determine if these viruses are the cause of the observed symptoms in star jasmine is needed.

I believe this study has clinical implications and further research will be able to show if this undiscovered virus is the cause of the symptoms observed and to see the over destruction to the plants infected. Star jasmines or other types of jasmines in other parts of the world may also be displaying these symptoms, which could gain from this discovery. In India and other countries, jasmine are highly valued for making tea, perfumes and used in religious and marriage ceremonies and jasmine also has medicinal properties used to treat diseases and ailments; this research can help keep their crops healthy. It is also important that the gene bank be updated with this new virus to help other researchers as they identify viruses.

Breakout Session IB: Development & Reproductive Biology / 95

The Role of SYM-3 and SYM-4 in Tissue Integrity and Organogenesis

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sym-3 and sym-4 encode structurally unrelated but highly conserved proteins. Mutations in sym-3 and sym-4 are synthetically lethal with mec-8, which encodes a conserved RNA-binding protein involved In alternative splicing. mec-8;sym3/4 double mutants arrest as L1 larvae, whereas sym-3;sym-4 double mutants are viable, suggesting that sym-3 and sym-4 act together within a pathway or a complex. Mec-8;sym3/4 Mutants show defects beginning at the 1.5-fold stage of embryogenesis, at which time the anterior pharynx, having attached to the epithelium, begins to elongate. Whereas wild-type embryos show only a slight ingression at the anterior hypodermis in the region of future buccal cavity, mec-8;sym3/4 mutants display a strong deformation of anterior hypodermis, which resembles a keyhole. Subsequently, the pharynx and buccal cavity of mec-8;sym-3/4 L1 larvae are displaced towards the posterior, preventing feeding and leading to a “bulbous nose” phenotype. Using alleles of pha-1, we find that formation of the keyhole, displacement of pharynx, and formation of bulbous nose can be suppressed by preventing early pharyngeal attachment or by severing the connection between the elongating pharynx and the anterior hypodermis later in development. Our results provide evidence for an inward pulling force on the anterior hypodermis during pharyngeal elongation and suggest that the ability of the hypodermis to withstand this force is weakened in mec-8;sym-3/4 mutants. Interestingly, a keyhole defect is also observed in mir-51 family mutants, and this phenotype is also suppressed by pha-1 mutants. To understand the roles of sym-3, sym-4 and mec-8 in the maintenance of hypodemal integrity, we have undertaken RNAi- feeding screen for genes that interact with sym-3 and sym-4. We are also seeking to identify sym-3 and sym-4 binding partners using a 2-hybrid approach and to characterize the expression of sym-3 and sym-4. Finally, we describe a strategy for isolating genetic suppressors of mec-8;sym3/4 mutants using a counter-selectable marker.

Poster Session - Board 045 / 96

CHARACTERIZATION OF WEST NILE VIRUS-INDUCED MEMBRANE STRUCTURES REVEALS A NOVEL ROLE FOR NS1 PROTEIN IN INDUCTION OF VESICLE PACKETS FOR VIRAL RNA SYNTHESIS

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BACKGROUND: West Nile Virus (WNV) modifies intracellular membranes of the host cell, resulting in the formation of clusters of vesicle packets (VP) to establish its sites of replication. Although the role of VP for viral RNA synthesis is well documented, the WNV protein(s) responsible for induction of the VP has not yet been identified. In this study, we describe a novel role for NS1 in the induction of these VP structures during WNV infection. METHODS: HEK293T cells were infected with WNV New York 99 strain (NY99) and were analyzed for localization of NS1 protein in VP structures over time using immunofluorescence (IF) assay. WNV replication was determined using qRT-PCR and plaque assays, and NS1 protein expression was verified using western blot. To demonstrate induction of VP with only NS1 protein, we synthesized cDNA from WNV NY99 RNA using reverse transcriptase and amplified the entire NS1 gene, including the endogenous signal sequence from the WNV envelope structural protein, by high-fidelity PCR. To visualize expressed NS1 in transfected cells, green fluorescent protein (GFP) or V5 epitope harbored in the expression vector was fused to the C-terminal end of the NS1 gene. Plasmids were transformed into chemically competent E. coli (DH5α) cells and were isolated, purified and sequenced. The resulting plasmids were used for expression of NS1 protein in transfected HEK293T cells. The VP structures induced by only NS1 were analyzed using transmission electron microscopy (TEM). RESULTS: Using high-resolution confocal microscopy, the NS1 protein was found to localize to the VP, which appeared as fluorescent particles (FPs) scattered in the cytoplasm along with replicating viral RNA. Toward the end of the eclipse phase of infection, majority of the FPs aggregated at the perinuclear region. The aggregation of the FPs coincided with increase in viral RNA copies and virion production. TEM data using NS1 transfected cells revealed that the NS1-induced membrane structures were similar to VP structures observed in infected cells indicating that NS1 protein is responsible for VP induction. CONCLUSION: NS1 has previously been implicated in viral RNA replication and results from this study suggest that NS1 may also play a role in VP induction. Studies are underway to investigate the function of NS1 in the WNV life cycle, explore the sequential events in VP biogenesis, and determine whether host proteins are involved in VP formation and expansion.

Breakout Session IIA: Cardiovascular / 97

Beating without oxygen: Mechanisms of bradycardia during prolonged anoxia in the anoxia-tolerant freshwater turtle (Trachemys scripta)

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Cardiovascular disease, which is characterized by a reduced blood supply and therefore oxygen supply to the heart muscle, and which usually culminates in a heart attack, is the primary cause of death in the U.S. Understandably, medical science has been feverishly attempting to discern how to counteract anoxic (no oxygen) heart damage. However, nature has already solved the problem. There exists a few vertebrate species that have the remarkable capability to survive without oxygen for hours, days and even months, during which time their heart continues to beat rhythmically. Among them, the freshwater turtle (Trachemys scripta) is undoubtedly one of the most impressive examples. The turtle overwinters in ice-covered ponds, which become progressively hypoxic (low oxygen), and ultimately anoxic, as thick ice coverage inhibits both photosynthesis and oxygen diffusion from the air. During anoxia, turtle enters a severe hypometabolic state, and correspondingly, cardiac activity is massively reduced. At cold temperature, the heart contracts less than once a minute. My research has investigated how the bradycardia is brought about, and has revealed that the slowing of heart rate is mediated by a number of extrinsic and intrinsic factors, including alterations in cardiac autonomic control, modified cardiac electrophysiology and altered myocardial high-energy phosphate metabolism. Recent research at the cellular and molecular level has revealed that key components of the cardiac "pacemaker" are differentially expressed at the transcript level with anoxia exposure and with cold acclimation in a manner that may facilitate the severe down-regulation of cardiac activity during anoxia. Continued investigations on how the heart of anoxic turtles regulated during prolonged anoxia exposure and is capable of beating in rhythmic, albeit reduce manner promise to expand our mechanistic comprehension of how the vertebrate heart can function in the presence of little or no oxygen.

Breakout Session IC: Infectious Diseases - Parasites & Vectors / 98

Protein Modifications Used by Parasitic Nematodes to Evade the Host's Immune Response: A New Approach by Metabolic Labeling in C.elegans

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Background: Pathogens modify proteins and lipids by phosphorylcholine (Pc). This alters the host-cell function and helps to evade the host immune system. Parasitic nematodes synthesize Pc-modified biomolecules that can modulate the host's antibody and cytokine production to favor nematode survival, contributing to long-term infections. Only two nematode Pc-modified proteins have been unequivocally identified, yet discovering the protein targets of Pc modification will be paramount to understanding the role(s) that this epitope plays in nematode biology. A major block in the field has been the lack of techniques for selective purification of Pc-modified proteins. The non-parasitic nematode Caenorhabditis elegans expresses Pc-modified N-linked glycans, offering an attractive model to study the biology of Pc-modification.

Objective: To develop a chemical biology approach to detect, purify and identify C. elegans Pc-proteins with high specificity.

Methods: We developed a robust method to identify Pc-modified proteins that employs metabolic labeling of primary embryonic C. elegans cells with propargylcholine, an alkyne-modified choline analog. A Click chemistry reaction between the terminal alkyne of propargylcholine and biotin-azide using a copper Cu(I) catalyst enabled streptavidin purification, high-throughput liquid chromatography and mass spectrometry identification of propargyl-labeled proteins.

Results:. All 21 proteins identified using stringent criteria are known or predicted to be membrane or secreted proteins, consistent with the model of a Golgi-resident, putative Pc-transferase. Of the 57 Pc-N-glycosylation sites reported, 35 have been previously observed as N-glycosylation sites in high-throughput screens of C. elegans. Several identified Pc-proteins are nematode-specific proteins, but nine of the Pc-modified proteins are widely conserved ion transporters and amino acid transporters, while 12 others are conserved proteins involved in synaptic function.

Discussion and Conclusions: This work provides a method to identify Pc-modified proteins in C. elegans and related nematodes to further our understanding of the pathophysiological role of Pc. Our approach allows affinity purification of Pc-proteins from complex protein samples in the presence of harsh detergents and chaotropic agents, enabling the identification of low abundance membrane Pc-proteins. Several of the identified Pc-modified proteins are localized within intracellular compartments, suggesting a functional role for Pc-modification beyond immunomodulation. Interestingly, the incidence of auto-immune and inflammatory diseases, such as type I diabetes and multiple sclerosis, are reduced in areas of endemic nematode infection. Thus, Pc-proteins are being investigated for their therapeutic potential in treating inflammatory and auto-immune disorders.

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Poster Session - Board 060 / 99

Multiple proteins with essential mitochondrial functions have glycosylated isoforms

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Background: O-linked N-acetyl-glucosamine glycosylation (O-GlcNAcylation) is an abundant, reversible and highly dynamic post-translational protein modification that regulates signal transduction, apoptosis, proteasome activity, transcription, translation and nuclear transport. Mitochondrial dysfunction underlies the pathology of several diseases including Alzheimer's disease and diabetes. O-GlcNAcylation of mitochondrial proteins is increasingly being recognized as a regulatory mechanism contributing to these pathologies. Deregulated O-GlcNAcylation has been linked to diabetes-related complications, cancer progression, neurodegeneration and includes mitochondrial dysfunction. Nucleocytosolic, secreted, and membrane proteins are commonly modified by glycosylation but reports of glycosylated mitochondrial proteins are rare.

Objective: We sought to determine whether the glycosylation of proteins with established mitochondrial function might be more common than previously appreciated.

Methods: We employed lectin affinity chromatography on enriched bovine heart mitochondria. In an independent approach, the glycosylation status of PDH E1 alpha was assessed by metabolic labeling of COS-7 cells with peracetylated azido-GalNAc, reacted via Click Chemistry with biotin-alkyne. The biotinylated, azido-labeled proteins were then purified by avidin enrichment and probed by Western blotting. To broadly investigate the glycosylation of proteins with known mitochondrial function, we performed, what is to our knowledge, the first high-throughput proteomics study aimed at identifying glycosylated isoforms of proteins with mitochondrial function.

Resuls: We have identified glycosylated isoforms of five proteins with classic mitochondrial function by using a candidate approach. These are: Pyruvate dehydrogenase E1 alpha-subunit (PDH E1 alpha), ADP/ATP translocase (ANT), Complex I subunit NADH dehydrogenase [ubiquinone] iron-sulfur protein 3 (NDUFS3), and three subunits of ATP synthase: subunit alpha, subunit d, and the oligomycin sensitivity conferring protein (OSCP). Using three complementary approaches for glycoprotein enrichment and detection, we identified glycosylated isoforms for 88 proteins with mitochondrial function, of which 67 are novel.

Discussion and Conclusions: Our study shows that glycosylated isoforms of these mitochondrial proteins constitute a small fraction of the total protein pool. This finding may explain the low frequency with which such glycosylated isoforms are identified in large-scale studies. It also suggests that glycosylation of such proteins could potentially play a regulatory role. Our identification of candidate glycosylated isoforms for 88 proteins with mitochondrial function, of which 67 are novel, supports our earlier conclusion that glycosylation of such proteins is more common that has been historically appreciated. Our data suggests that glycosylation of these proteins plays an unexplored role in the biology of mammalian cells. This role may include regulating these proteins' function, stability, or subcellular localization, as has been shown for other glycoproteins.

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Poster Session - Board 044 / 100

Mechanisms underlying dengue virus-induced vascular leakage and mononuclear cells migration across human endothelial cells transwell permeability model

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Background: Dengue virus (DENV)-infected patients who progress to dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS) have hallmark signs of increased vascular permeability and plasma leakage. While DHF/DSS patients demonstrate increased levels of chemokines and cytokines and perivascular infiltration of mononuclear cells, specific mediators that trigger vascular permeability and the precise mechanisms involved in mononuclear cells infiltration are unclear.

Objective: We previously demonstrated that endothelial cells increase expression of adhesion molecules, ICAM-1 and VCAM-1, upon treatment with supernatants from DENV-infected monocytes (Virology 2012;422:326-337). In this study we characterized DENV-infected peripheral blood mononuclear cells (PBMCs) and measured their potential to alter endothelial cell permeability, adhesion and transmigration. We hypothesized that DENV-infected PBMCs increase expression of lymphocyte function-associated antigen 1 (CD11a) and macrophage-1 antigen (CD11b), receptors for ICAM-1 and VCAM-1, respectively.

Methods: PBMCs were isolated from fresh whole blood and either infected with DENV, treated with TNF-alpha or mock-treated. Flow cytometry was employed to measure CD11a and CD11b expression. A Transwell® microvascular endothelial cell permeability model developed in our laboratory was infected with DENV, treated with TNF-alpha, mock-treated and employed to identify changes in transendothelial electrical resistance (TEER) and transmigration after incubation with PBMCs. Endothelial cell monolayers infected with DENV, treated with TNF-alpha or mock-treated were employed to measure adhesion of PBMC. The CytoSelect® leukocyte-staining assay allowed for fluorescent readout of adherent and transmigrated PBMCs.

Results: We demonstrated by flow cytometry that approximately 76% of DENV-infected PBMCs expressed CD11a and CD11b compared to approximately 65% and 55% of mock-treated PBMC, respectively. When incubated in the Transwell® model, DENV-infected PBMCs reduced TEER by 25% and increased migration by 50% compared to mock-treated controls at 48 hours after infection. DENV-infected PBMCs increased adhesion to endothelial cells by approximately 30% compared to mock-treated controls. Moreover, TNF-alpha treated PBMCs decreased TEER and increased expression of CD11a, CD11b, adhesion and migration to levels greater than DENV-infected PBMCs.

Conclusion: Increased expression of CD11a and CD11b found on DENV-infected PBMCs may be important for elevated vascular permeability and increased mononuclear cell migration observed in DHF/DSS patients. Also, our results support clinical observations of a "cytokine storm" (including TNF-alpha) contributing to increased vascular permeability. Future experiments will attempt to reverse the effects of DENV infection by using monoclonal antibodies against adhesion molecules or their mononuclear cell receptors as possible therapeutics.

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Breakout Session IIIA: Infectious Diseases - Viruses / 101

Recombinant Filovirus Antigens are Safe and Potent Immunogens for Inducing Cellular and Humoral Immunity in Rodents and Non-human Primates and Provide Protection against Lethal Live Virus Challenge

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Ebolavirus and Marburgvirus cause fulminant hemorrhagic fevers, with a case-fatality rate of up to 90%, and have been identified as high priority bioterrorism threats. No FDA approved antiviral treatments or vaccines are currently available.

To develop a recombinant subunit vaccine, soluble recombinant Filovirus surface glycoproteins (GP) and matrix proteins (VP24 and VP40) were generated in the Drosophila S2 cell expression system and purified by immunoaffinity chromatography. The immunogenicity of individual recombinant Zaire ebolavirus (ZEBOV) subunits and admixtures formulated with or without clinically relevant adjuvants was evaluated in mice, guinea pigs and macaques.

Strong antigen-specific IgG responses were detected by ELISA in all species after administration of two or three doses of adjuvanted formulations. In addition, Ebola virus neutralization was observed. In mice and non-human primates subunit proteins were shown to elicit cell mediated immune responses, as significant B- and T-cell stimulation was observed in immune lymphocytes after antigen re-stimulation. Analysis of secreted cytokines in batch-cultured, antigen-stimulated splenocytes or PBMC's demonstrated antigen-induced Th1 and Th2 type responses.

Vaccine candidates formulated with or without adjuvant were tested in mice for direct protection against challenge with mouse-adapted ZEBOV. All vaccine formulations containing ZEBOV GP generated protective responses and serum transfer from such animals into naïve mice demonstrated that humoral immunity alone can be fully protective. Furthermore, the transfer of immune splenocytes into naïve mice showed that recombinant GP and VP24 subunits elicit functional T cell responses that lead to protection against live virus challenge.

Immunogenicity and efficacy studies in guinea pigs were focused on optimized antigen dosing, antigenic balance and adjuvantation. Various lead formulations consistently produced high antibody responses and demonstrated 100% protective efficacy in the ZEBOV guinea pig model. In macaques, preliminary results suggest that vaccination with GP+VP40+VP24 and an emulsion-based adjuvant may prevent viremia subsequent to live virus challenge and therefore protects animals from terminal ZEBOV disease.

The completed studies suggest that a viable filovirus vaccine candidate based on non-replicating viral subunits has been identified. Further preclinical formulation optimization will be aimed at selecting a fully protective candidate with multi-valent efficacy profile in non-human primates that can advance into future clinical testing.

Breakout Session IIIB: Cell & Molecular Biology / 102

Differential expression profile of microRNA and neuroinflammatory target mRNA in diabetic mouse brain

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Background: Enduring cognitive deficit and diabetic encephalopathies are widely recognized complications among type 2 diabetes (T2D) patients. The complex underlying mechanisms require an extensive understanding of cellular and molecular events to identify modifiable therapeutic targets. MicroRNAs (miRNAs) are considered key regulators of gene expression during normal tissue development as well as metabolic and inflammatory pathways in T2D. They modulate both physiological and pathological pathways by post-transcriptionally inhibiting expression of their target genes.
Objective: The objective of this study was to investigate the influence of diabetes on expression levels of miRNAs and their neuroinflammatory targets in the brain.
Methods: Genome-wide miRNAs expression in C57BL/6J (WT) and db/db mice was analyzed using quantitative real-time polymerase chain reaction (qRT-PCR) based miRNA PCR Array. Similarly, mRNA expression of key pro- and anti-inflammatory molecules in the same brains was also analyzed using mRNA PCR arrays to examine whether the differentially expressed miRNAs could regulate their target genes. Data was analyzed using RT² Profiler PCR Array Data Analysis version 3.5 and Ingenuity Pathways Analysis (IPA).
Results: Our data indicate that among the 528-mouse miRNA represented on the chip, a total of 136 miRNA were differentially expressed (by > 2-fold) in the brains of db/db mice as compared to WT control brains. IPA demonstrated that these miRNAs and their targeted genes have been indicated to play a role during brain development, neuroinflammation and neurodegeneration. Moreover, using same mice brain samples, we demonstrated an inverse correlation between the miRNAs expression and their target mRNA genes. Up-regulation of specific miRNA such as let-7, miR-27b, miR-155 and miR125b-5p as well as the down regulated miRNAs such as miR-196a and miR-20b was correlated with their downstream mRNA targets, such as functional TGF-β mRNA targets, and inflammatory chemokines, cytokines and transcription factors in the diabetic mice brain.
Conclusions: This study for the first time demonstrates that diabetes modulates multiple miRNAs expression in the mouse brain, which may control gene expression of pathways that may play role in diabetes-associated cognitive deficit and encephalopathies. Further studies are warranted to identify the functional roles of these miRNAs in modulating neurological functions during diabetes such as memory, learning, cognition, neuroinflammation and neurodegeneration.
Funding: Institutional funds and grant (P20GM103516) from the Centers of Biomedical Research Excellence (COBRE), National Institute of General Medicine, National Institutes of Health. We thank Dr. Gordon Okimoto and Mr. Mike Loomis of University of Hawaii Cancer Center and COBRE Bioinformatics Core for assistance with Ingenuity analysis.

Breakout Session IIC: Infectious Diseases - Bacteria & Fungi / 103

Identification of virulence factors in the bacterium Burkholderia pseudomallei

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Background: The select-agent bacterium Burkholderia pseudomallei possesses a large repertoire of known and unknown virulence factors that culminate in the infection known as melioidosis.

Objective: The objective of the research is to identify unknown virulence genes that contribute to the cellular infectious process through a genome-wide screen.

Methods: A comprehensive transposon mutant library was constructed from an engineered strain of B. pseudomallei and used to infect RAW264.7 murine macrophages at two different MOIs. The transposon/genome junctions were enriched for TNseq then sequenced using Illumina sequencing. Between 6 and 10 million reads per sample were obtained and mapped using Bowtie. HTSeq python scripts were used to count the reads per gene, which were visualized using IGV (Integrative Genome Viewer 2.3) and analyzed using MeV (Multi-Experiment Viewer). Bacterial genes that were not present in the samples after serial passaging through the macrophages were considered putative virulence genes. Deletion mutants for the genes were obtained and verified for an inability or decreased ability to infect RAW macrophages.

Results: 901 genes were identified as being 10-fold negatively selected against. 333 of those genes were 100-fold negatively selected against and 113 were 1,000-fold negatively selected against. Several loci already associated with virulence were identified, including genes of the T6SS-1, Bsa (Burkholderia secretion apparatus T3SS), flagella, and LPS biosynthesis; lending confidence that the data is reliable. DAVID gene ontology analysis of the gene list indicated enrichment for the term pathogenesis. Numerous loci encoding secondary metabolite biosynthetic genes and transcriptional regulators were also enriched for. Several gene knockout mutants were used to infect macrophages and showed a decreased ability to cause infection, agreeing with the TNseq data.

Conclusion: TNseq is a powerful tool that allowed the identification of previously unknown virulence factors that contribute to the ability of B. pseudomallei to infect cells. Virulence regulators that allow B. pseudomallei to react to cellular defenses and eventually overtake them were also found. Identification of the genes provides novel targets for therapeutics and increases our understanding of the arsenal that B. pseudomallei utilizes to overcome host defenses.

Poster Session - Board 055 / 104

Chronic Insulin Exposure Induces Ectopic Lipid Accumulation and ER-reprogramming in Mast Cells

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Background: We previously showed that mast cells (MC) chronically exposed to insulin respond by developing steatotic levels of cytosolic lipid bodies, suggesting that immunocytes, like hepatocytes and myocytes, are sites of lipid sequestration in response to dysregulated insulin levels. This ectopic lipid accumulation influences mast cell functionality, biasing mast cell phenotype towards production of bioactive lipid mediators (LTC4) and away from release of histamine and other secretory granule components.

Objective: Our study presents an analysis of whole cell and lipid body lipidome in control, and insulin-exposed mast cells.

Methods: RBL2H3 and BMMC cells were used. Cells lysed and resolved by SDS-PAGE, electro-transferred to PVDF, and incubated with primary antibodies; developed with antibodies comprised of anti-rabbit or anti-mouse IgGs conjugated to horseradish peroxidase (Amersham). Signal visualized via Western blot. Cells grown and stained with Oil Red O, LipidTOX, Laurdan, Prodan, Filipin, DPH, and Nuclear stain DAPI. Bright field and fluorescence imaging performed on Nikon Ti Eclipse system and analyzed in NIS Elements. RBL2H3 treated and stimulated via FceRI or PMA/ionomycin. LTC4 assayed using Cayman Chemical's EIA kit. Lipids extracted by Folch et al methods. Neutral lipid and individual phospholipid classes was separated by liquid chromatography. Each lipid class was separated and quantified by gas chromatography. Lipidomic analysis performed in collaboration with Metabolon Inc. ER isolation performed using a Sigma kit. Cytochrome C reductase assay kit used to confirm ER enrichment. Proteome analysis (DIGE) performed for protein samples derived from control and 6d Insulin-FDI treated RBL2H3. RBL2H3 incubated with 1uM Fluo-4 AM. Assay performed in either 1mM Ca or Ca Free + 1mM external solution and stimulated. Calcium signals acquired using a Flexstation 3 and data analyzed using SoftMax® Pro 5. Electron microscopy performed using uranyl acetate/Pb and images quantified using NIH Image J.

Results: Our data shows a significant upregulation in lipid-associated pro-inflammatory precursor molecules in response to chronic insulin exposure. We also show the lipid body population in these cells is heterogeneous to a previously unsuspected degree. Moreover, due to the intimate relationship between Endoplasmic Reticulum (ER) and lipid body production, we tested the hypothesis that the ER may be altered in response to chronic insulin. Indeed, our data show that (in a manner analogous to observations in hepatocytes from obese models) the ER is reprogrammed towards a lipogenic phenotype, is morphologically distended, is compromised as a calcium store and exhibits certain indicators of a Unfolded Protein Response (UPR)/ER stress response in response to chronic insulin.

Conclusion: Collectively, this data shows that chronic insulin exposure in a model mast cell system drives lipidomic remodeling in a manner that alters lipid body formation and mast cell pro-inflammatory function.

Poster Session - Board 049 / 105

Detecting the prevalence of Taro bacilliform virus in Pacific Taro varieties

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Taro (Colocasia esculenta), or kalo, is an important staple crop in the Hawaiian culture and economy. However, production of Taro products has declined recently due to environmental stresses but also suffers from some pests and diseases, one of which is the Taro bacilliform virus (TaBv). This is a double stranded DNA pararetrovirus also known as a badnavirus. These viruses are able to integrate into the host genome and replicate by the host machinery. We screened 12 varieties to detect the incidence of infection by TaBv. Infected taro plants were further investigated to see whether the viral genome is integrated into these Taro genomes or is episomal. Fully emerged leaves from a Taro germplasm repository maintained at the Pearl City Urban Garden Center were collected and DNA extraction was performed using a Puregene extraction kit. PCR was done to screen these varieties for the Badna virus using TaBv 1 and TaBv 4 primers, then visualized on an agarose gel. The genomic DNA was used for Southern blotting with a DIG-labeled probe designed from a sequenced Badna plasmid. The PCR showed over 90% of the varieties tested positive for the virus. Faint bands on the Southern blot at approximately 23 kb suggested seven varieties have an integrated Badna virus, while one variety may have an episomal Badna virus due to the lower molecular weight of its signal. Due to the low signal to noise ratio of this blot, additional varieties need to be screened for conclusive evidence of integration.

Breakout Session IIA: Cardiovascular / 106

Signaling Pathways Regulating Cardiac Fibroblast Development and Function

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Background: The basic helix-loop-helix (bHLH) family of transcription factors orchestrates cell fate specification, commitment, and differentiation in multiple cell lineages during development. Here, we describe the role of a bHLH transcription factor, Tcf21 in specification of the cardiac fibroblast lineage. In the developing heart, the epicardium constitutes the primary source of progenitor cells that form two cell lineages, coronary vascular smooth muscle cells (cVSMCs) and cardiac fibroblasts. These fibroblasts constitute two types, those that are scattered throughout the ventricular wall (interstitial) and those that deposit matrix around blood vessels (adventitial).
Objective: Currently, there is a debate regarding if the specification of the smooth muscle cells and the fibroblasts occurs early in the formation of the epicardium or later after the cells have entered the myocardium. Our goal was to determine when specification of cardiac fibroblasts occurs.
Methods: We performed lineage tracing using a tamoxifen-inducible Cre expressed from the Tcf21 locus in the mouse during development and in the adult.
Results: We found that the majority of Tcf21 expressing epicardial cells are committed to the cardiac fibroblast lineage prior to initiation of epicardial epithelial-to-mesenchymal transition (EMT). Furthermore, Tcf21 null hearts fail to form cardiac fibroblasts, and lineage tracing of the null cells showed their inability to undergo EMT. We are now performing cell tracing experiments in several models of heart disease to determine the differential role that these endogenous fibroblasts play during each of the models. Preliminary data reveals that the Tcf21 population of cells expands in each model, and gene expression profiling will be examined to ascertain changes in gene induction for this population of cardiac fibroblasts.
Conclusion: This is the first report of a transcription factor essential for the development of interstitial and adventitial fibroblasts of the heart. We show a unique role for Tcf21 in multipotent epicardial progenitors prior to the process of EMT that is essential for cardiac fibroblast development.

Poster Session - Board 018 / 107

Dietary Analysis in Regard to Polycystic Ovarian Syndrome

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Dietary Analysis of Fatty Acid Intake In Reference to Polycystic Ovarian Syndrome

Background: Polycystic Ovarian Syndrome (PCOS) is an inflammatory endocrine disorder which afflicts women, characterized by ovarian cysts and is noted for inducing infertility in women, along with increasing testosterone levels, which lead to a variety of superficial issues including persisting acne and hair loss. Recent findings have shown PCOS to have a high correlation with the onset of type 2 diabetes; a correlation as high as over 50%. In regards to inflammation, omega-6 (n-6) polyunsaturated fatty acids (PUFA) have recently been shown to induce inflammatory responses in lipid membranes of cells, along with saturated fats which have always been known to do so. On the contrary, omega-3 (n-3) PUFA are known to be anti-inflammatory, and are in fact used to treat inflammatory disorders, including rheumatoid arthritis.

Objective: To observe the diets of both females with and without PCOS in regard to their saturated fat, n-6 PUFA and n-3 PUFA consumption in order to determine if a correlation of elevated consumption of the saturated fats and n-6 PUFA are found in the women diagnosed with PCOS.

Methods: Two groups of women, one diagnosed with PCOS and one not, were submitted to food frequency intake forms which featured the consumption of any food containing lipids of relevance to this study. The values were computed to a daily average and compared between groups.

Results: The study showed a greater consumption of n-6 PUFA (7.627 +1.864 versus 5.048 +0.870 g/day) and saturated fats in the non-PCOS group (10.589 +1.897 versus 8.854 +.962 g/day), a greater amount of n-3 PUFA consumed in the PCOS group (120.332 +31.62 versus 81.923 +33.746 mg/day), and a higher consumption ratio of n-6:n-3 PUFA in the non-PCOS group versus the PCOS group (7.529 +.833 versus 6.018 +1.195).

Conclusions: One plausible explanation for these data is that once these women were diagnosed with PCOS and were made aware of the dangers as a result of PCOS onset, they began to select healthier dietary choices. On the contrary, those women who do not have PCOS have had no reason to change their diet, and thus still have elevated levels of saturated fat and n-6 PUFA intake. What may be the root of PCOS onset in regards to dietary choices, and fatty acid intake in particular, is those choices that are made around the time of the onset of puberty in females.

Poster Session - Board 026 / 108

Requirement for PDGFRα in hepatic fibroblasts

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Background: Liver damage and scarring, caused by alcoholism, nonalcoholic steatohepatitis, or hepatitis, can lead to fibrosis and eventually cirrhosis. Cirrhosis has been recognized as the 12th leading cause of death in the US. There is no known cure for liver cirrhosis, and current treatments are limited to prevention of additional damage until a liver transplant becomes necessary. The cells believed to be responsible for the fibrosis are fibroblasts, but little is understood about this population of cells. A better understanding of the signals controlling fibroblast differentiation will provide novel information about the disease process of liver fibrosis.
Objective: Our lab has previously shown that knockout mice for both the transcription factor TCF21 and the receptor tyrosine kinase, PDGFRα, completely lack cardiac fibroblasts. This has led to the hypothesis that TCF21 and PDGFRα could be essential genes for fibroblasts in other organs. The aim of this study was to investigate the development and gene expression of the liver fibroblast population with specific emphasis on TCF21 and PDGFRα expression and function.
Methods: Mice that permit tracing of different cell populations were used to understand the formation of the liver fibroblast populations during embryonic and postnatal development. The gene expression profiles of these cells was analyzed in vivo and in vitro using immunohistochemistry, qPCR and western blotting on both tissue samples and in primary cell cultures.
Results: We have found that in the adult liver the TCF21 lineage expresses PDGFRα and that loss of PDGFRα and PDGFRβ signaling leads to a reduction in the numbers of these cells. Further analyses indicate that this TCF21 lineage has an expression profile consistent with what is known about hepatic stellate cells.
Conclusion: Results from these experiments provide important insights into origin and signaling requirements of the liver fibroblast population. A clear understanding of the development of these cells, specifically with regard to TCF21, will permit us to elucidate possible signaling pathways which can be manipulated to attenuate liver fibrosis and prevent cirrhosis.

Poster Session - Board 028 / 109

Cardiac fibroblasts regulate the endothelium

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Cardiac fibrosis is characterized by progressive deposition of extracellular matrix (ECM), eventually leading to cardiac failure. Epicardial-derived cardiac fibroblasts have been suggested to play a major role in cardiac fibrosis through the remodeling of the ECM. These fibroblasts require stimulation of platelet-derived growth receptor alpha (PDGFRα) for persistence and proliferation, and specific deletion of PDGFRα in Tcf21-expressing yields a dearth of cardiac fibroblasts. The deletion in this lineage of cells affects not only the ECM, but the cardiac vasculature as well. Inflammation and invasion of leukocytes play a vital role in the pathogenesis associated with heart injuries, and it hypothesized that cardiac fibroblasts assist in modulating vascular permeability and angiogenesis in the diseased heart. In this study, we analyze vessel number, diameter, and permeability in healthy and diseased hearts lacking a specific population of epicardial-derived cardiac fibroblasts. These results will assist in defining the roles of cardiac fibroblasts and their contribution to the healthy and diseased heart, furthering our capability of human treatment.

Poster Session - Board 019 / 110

Identification of Novel Tcf21 Downstream Targets

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Background: Pathological fibrosis in the heart is caused by the excessive proliferation and deposition of extracellular matrix, primarily by cardiac fibroblasts. A basic helix loop helix (bHLH) transcription factor necessary for development of the cardiac fibroblast population isTcf21. Previous studies from our lab have shown that a Tcf21 knockout mouse model results in an absence of cardiac fibroblasts.
Objective: The objective of this project is to elucidate the genes directly downstream of Tcf21, with specific focus on those with a role in cardiac fibroblast development and/or differentiation.
Methods: Previously our lab has completed deep-sequencing on Tcf21 null embryonic hearts in comparison to wild type at E13.5 (embryonic day 13.5) and E18.5. The resulting list of genes was narrowed to include only those which showed significant fold difference between the two samples. We have validated changes in levels of expression in several genes from this list by quantitative PCR (qPCR) at the E13.5 time point. Cis-regulatory regions from genes that had significant fold difference in the qPCR will be further analyzed by chromatin immunoprecipitation (ChIP) to ascertain direct binding by Tcf21.
Results: We have performed ChIP in the lab using both a commercially available Tcf21 antibody as well as a polyclonal Tcf21 antibody generated by our lab. Several targets have been identified by the deep-seq with promoter sequences containing CANNTG sites (which are known binding sites for the bHLH transcription factor family) and these loci will be analyzed for Tcf21 binding.
Conclusion: Results from this project will allow us to identify Tcf21 targets and give us a better perspective on cardiac fibroblasts. Future experiments include ChIP-sequencing to obtain a comprehensive list of cis-regulatory elements bound by Tcf21 in the embryonic mouse heart.

Breakout Session IIA: Cardiovascular / 111

Hypoxia Inducible Factor regulates cardiac RNA splicing

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Background: Ischemic cardiomyopathy is associated with elevated levels of hypoxia inducible factor (HIF)-1, a transcription factor that plays a key role in the response to hypoxia.
Objective: The objective of this study is to elucidate the role of CaMKIIγ splice variants in regulating the response to hypoxia.

Methods: We have created a transgenic mouse model with inducible cardiomyocyte-specific expression of an oxygen-stable form of HIF-1α to better understand the role of HIF-1 in ischemic cardiomyopathy. We performed RNAseq on polyA selected cardiac RNA before and after three days of transgene expression, with 10 million processed reads per sample. Genes were considered possibly differentially expressed if, following adjustment for multiple testing, p-value for the comparison to uninduced was less than 0.05. Genes having evidence of alternative splicing (those with a q-value <0.05 suggesting a change in the relative abundance of the different transcripts deriving from a single transcription start site) were identified.

Results: A total of 22 alternatively spliced genes were investigated. One transcript in particular, that for Calmodulin-dependent protein kinase II isoform gamma 2 (CaMKIIγ) was of particular interest because CaMKII homologs are involved in the phosphorylation of both RyR2 and PLB, and might thus be important in excitation–contraction coupling and cardiac contractility. The reference RNA sequence for CaMKIIγ was down regulated 11.7-fold, while variant 1 (missing Exons 7 and 8), and variant 2 (missing Exon 8) were up regulated 2.6-fold and 1.7-fold, respectively. Real-time PCR validation of the RNAseq data has been obtained in the transgenic hearts, and, to a lesser extent, in wild type mice subjected to LAD ligation.

Conclusion: The biochemical effect(s) of this variation in splice variants remains unknown, but seems likely to be connected to the decrement in cardiac contractility seen with HIF expression, and in ischemic cardiomyopathy.

Breakout Session IIA: Cardiovascular / 112

Role of an mTOR-stabilizing protein Tel2 in cardiomyocytes

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Previously we showed that the expression level of the mechanistic target of rapamycin (mTOR), a key mediator of cell growth and cell survival, increases in patients with heart failure. We also demonstrated that cardiac mTOR is sufficient to prevent cell death and protect the heart against ischemic stresses, including myocardial infarction. However, a mechanism of regulating mTOR expression in the heart remains unknown. Recent studies demonstrated that telomere maintenance 2 (Tel2) stabilizes mTOR in other tissues. To assess the role of Tel2 in cardiomyocytes (CMs), we examined whether Tel2 is sufficient to stabilize and activate cardiac mTOR function, and prevent cell death against hypoxic stress. We overexpressed Tel2 in CM HL-1 cells by lipofectamine. Mock transfection was used as a control. 24 hrs later, CMs were subjected to 24-hour hypoxia. Cell survival was assessed by morphological changes, and the histone-associated DNA fragmentation (cell death ELISA). To study the mTOR signaling pathway, we examined the total expression levels of Tel2 and mTOR, and phosphorylation levels of S6 (mTORC1 substrate) and Akt (mTORC2 substrate) by Western blot. Overexpression of Tel2 significantly increased the level of mTOR expression. Phosphorylation levels of both Akt and S6 were much higher in Tel2–overexpressed CMs than controls, suggesting that Tel2 activates both TORC1 and TORC2. The round-shape CMs, indicating cell injury, were less in Tel2-overexpressed CMs compared to controls. The assessment of fragmented DNA by Cell Death ELISA showed hypoxia-induced apoptosis was increased by 4-fold increase in hypoxic control ($p<0.01$, $n=3$) compared to normoxic control, whereas Tel2 overexpression suppressed apoptosis by almost 2 fold increase compared to hypoxic control ($p<0.05$, $n=3$). These findings suggest that Tel2 is sufficient to protect CM against hypoxia, at least in part via mTOR activation. A study of Tel2-mediated mTOR activation would provide a novel therapeutic target for a therapy of heart failure following myocardial infarction.

Breakout Session IIIA: Infectious Diseases - Viruses / 113

Type I IFN-regulated IL-13 signaling alters susceptibility to post-influenza superinfection with MRSA

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BACKGROUND: Bacterial infections secondary to influenza infection are responsible for significant morbidity and mortality worldwide. Others have shown in murine models that 5-8 days after influenza challenge the susceptibility to secondary bacterial infections increases; though the mechanism of this increased susceptibility is still elusive. Here, we report that 2-3 days after influenza challenge (before the onset of clinical symptoms) mice had increased initial resistance to methicillin-resistant Staphylococcus aureus (MRSA)-induced pneumonia, but that superinfection with MRSA at that time during influenza infection resulted in exacerbated viral disease and death of mice.
OBJECTIVE: The objective of this study was to determine the mechanism of initial increase in resistance to post-influenza MRSA pneumonia that occurs during pre-clinical stage of influenza infection.
METHODS: Wild type and various knockout mice were first intranasally infected with mouse-adapted influenza virus A strain (A/PR8/8/34; H1N1), and were subsequently superinfected with MRSA (USA300, LAC). Intratracheal injection of recombinant cytokines and intraperitoneal injections of neutralizing antibodies were performed to determine the mechanism of resistance to MRSA challenge. Viral and bacterial burdens, and lung cytokine profiles were evaluated.
RESULTS: In the first 3 days of influenza infection mice had increased resistance to MRSA superinfection, requiring both type I IFN and IL-13 to inhibit IFN-γ production upon MRSA infection. IL-13 signaling induced early in influenza infection, exacerbated influenza pneumonia in wild type mice but ameliorated MRSA pneumonia in IFNAR-/- mice. At 7 days of influenza infection, mice had become highly susceptible to MRSA pneumonia, which was associated with increased IL-13Rα2 and IFN-γ production and was reversible with mrIL-13 or anti-IL-13Rα2 treatment.
CONCLUSIONS: Early in influenza infection IFNAR signaling increased IL-13 levels in response to MRSA challenge, which increased resistance to MRSA, but also susceptibility to influenza. The switch from increased resistance to increased susceptibility to secondary MRSA pneumonia during progression of influenza infection occurred as the capacity to produce IL-13 in response to MRSA challenge waned, IL-13Rα2 increased, and IFN-γ production increased in the mouse lungs. Thus, mechanisms of resistance to bacteria were not compatible with the mechanisms of resistance to the virus.

Poster Session - Board 036 / 114

Structural basis of RNA-guided target recognition and degradation by a bacterial surveillance complex

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Bacteria and archaea rely on CRISPR RNA-guided surveillance complexes for efficient detection of invading nucleic acids. In Escherichia coli small CRISPR derived RNAs (crRNA) are incorporated into a 405kDa ribonucleoprotein assembly called Cascade (CRISPR-associated complex for antiviral defense). Invasive dsDNA is identified and bound by Cascade through a mechanism that involves complementary base pairing to the crRNA and recognition of a specific dinucleotide sequence called a PAM adjacent to the DNA target. Once bound by Cascade, the helicase-nuclease Cas3 is recruited by an unknown mechanism to the foreign DNA resulting in strand destruction. Here we present our bioinformatic, biochemical and structural work aimed at describing the fundamental mechanisms of Cascade mediated strand invasion, PAM recognition, and Cas3 recruitment.

Breakout Session IIC: Infectious Diseases - Bacteria & Fungi / 115

The identification of the targets of the F-box protein Cdc4p in Candida albicans through proteomic analysis.

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Background: Candida albicans is a major fungal pathogen of immunocompromised humans, including those undergoing chemotherapy or tissue transplants and AIDS patients. C. albicans grows in both yeast and filamentous/hyphal forms and the switch to filamentous growth, with the corresponding changes in protein complement, are important for the virulence. Significant progress has been made in understanding the signaling pathways that control the morphogenesis switch and virulence. At least five negative and three positive regulatory pathways have been discovered, and many of the cell cycle regulators have also been shown to play a role in morphogenesis. However, the relationships between the signaling pathways, morphogenesis and the cell cycle is still not well understood. The ubiquitin ligases and their subunits, specifically the F-box proteins and SCF (Skp1/Cul1/F-box protein complex) ubiquitin ligases are traditional cell cycle regulators that catalyze the addition of ubiquitin peptides to "target" proteins. The F-box protein Cdc4p does not seem to play a critical role in cell cycle progression in C albicans, but is a negative regulator of filamentous growth.
Objective: In order to elucidate the mechanism through which Cdc4p regulates morphogenesis, we are identifying Cdc4p targets that then ubiquitinated by the SCF ligase complex.
Methods: Proteomic approaches are being used to identify the Cdc4p target protein.
Results: To date, we have identified 20 proteins whose levels are altered in cdc4/cdc4 mutants relative to wildtype cells with p-values less than 0.05 that are potential Cdc4p targets. These 20 proteins include Apr1 (a vacuolar aspartic proteinase), Cct5 (a T-complex protein 1 subunit), Cpr3 (a putative peptidyl-prolyl cis-trans isomerase), Cdc19 (a pyruvate kinase), Csp37 (hyphal cell wall protein), Ipp1 (a Putative inorganic pyrophosphatase), and Pfy1 (Profilin). To distinguish the changes in protein levels and post-translational modifications being a result of Cdc4p directly interacting with the putative targets or some indirect consequence of the deletion of cdc4, we are utilizing standard two-hybrid and western blots with anti-ubiquitin antibodies.
Conclusion: This project is increasing our understanding of the role post-translational modifications, such as ubiquitination, play in fungal pathogenesis.

Breakout Session IA: Neuroscience / 116

Analysis of neurotropic virus axon-to-cell spread

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Alphaherpes viruses are neurovirulent pathogens of the peripheral nervous system that include the human pathogens herpes simplex virus -1 (HSV-1) as well as the veterinary pathogen pseudorabies virus (PRV). These viruses infect their native hosts and invade the sensory neurons of the peripheral nervous system where they will establish latent infection. Spread between hosts occurs after viral reactivation when progeny virions are transported down sensory axons towards the peripheral mucosal epithelium resulting in characteristic lesions. We wanted to understand the events associated with the axon-to-cell spread that underlies this reactivation, particularly focusing on the population of virions that are associated with each event. Using a well characterized in vitro compartmentalized neuronal culture system and PRV and HSV-1 strains that express fluorescent fusion proteins we analyzed axonal egress at a single cell level in two complementary assays. Initially, we used a previously published method to determine the number of expressed genomes from a population of cells. Equivalent viruses that express one of three fluorophores fused to a nuclear localization sequence (XFP-NLS); mCeruelean, EYFP, or mRFP were generated for both PRV and HSV. The three viruses were used to infect compartmentalized neuronal culture and cellular fluorescence after axonal spread was imaged. This analysis demonstrated that a limited number of genomes are expressed from newly infected cells after axonal spread. To support this finding, we utilized a PRV strain that expressed a fluorescent capsids fusion protein to visualize virion egress. From this analysis, we found a limited number of capsids were localized within susceptible cells prior to nascent viral replication, suggesting the limitation on axonal spread is related to a restriction in the number of virions transmitted from axons. These complementary analyses indicate that the overall diversity of a viral population is limited after neuron to cell spread with a restriction at the point of axonal egress into the susceptible cell. These analyses are being extended into animal models and for other neurotropic viruses. This could represent a population bottleneck to viral diversity that could be targeted to eliminate spread between hosts.

Poster Session - Board 030 / 117

Effects of Iron Accumulation in a Model of Ischemic Myocardial Injury

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Heart failure affects approximately 600,000 Americans every year, and a variety of molecular mechanisms are thought to contribute towards the development of heart failure. Adverse left ventricular (LV) remodeling after acute myocardial infarction is an important factor for prognosis of subsequent development of heart failure. The mechanism of LV remodeling is not completely understood, but is thought to involve multiple processes such as prolonged inflammation and excess cell death. Recent reports indicate that iron accumulation in tissue injury impairs wound healing in the skin. However, the effects of iron accumulation in myocardial injury are not well characterized. We investigated whether iron accumulation occurs in murine models of ischemic injury, and the direct effects of iron molecules on cardiomyocyte viability. To study iron accumulation in ischemic injury, we subjected mouse hearts to an in vivo ischemia-reperfusion (I/R) model using left anterior descending coronary ligation (30 min transient ischemia). One week after in vivo I/R injury, the hearts were harvested for histological assays. Mason's trichome staining revealed that fibrotic scarring extended from the anterior to posterior wall in the midcardium. Iron staining showed significant iron accumulation along the scar area. Immunohistochemistry visualization showed that transferrin was highly expressed proximal to the scar areas. To investigate the potential toxic effect of excess iron, cardiomyocyte HL-1 cells were exposed to $FeCl_3$ in doses from 1 μM to 1 mM. After 24 hours of $FeCl_3$ treatment, cell death was assessed by a Live/Dead Cell Viability Assay (Invitrogen). Cells treated with 50 μM $FeCl_3$ or more exhibited significant cell death compared to controls, suggesting that increasing iron concentrations reduced cell viability and caused cell death in HL-1 cells. In conclusion, we observed and demonstrated iron accumulation in LV remodeling after I/R injury. We also demonstrated the direct toxicity of iron molecules on cardiomyocytes, further establishing links between excess iron accumulation and heart failure.

Poster Session - Board 020 / 118

Inhibition of ADAM10 and 8 reduces transendothelial migration of the monocytic cell line THP1 in vitro

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Metalloproteases of the A Disintegrin And Metalproteases (ADAM) family are important molecular mediators of inflammation. ADAMs participate at all stages of inflammation via the proteolytic cleavage of cell surface molecules. It has been shown that leukocyte expressed ADAM8 and ADAM17 are important during the initial rolling of leukocytes on the endothelium. However, the role of leukocyte expressed ADAMs in transendothelial migration and chemotaxis remains to be investigated and in particular it is still unclear to what extent ADAM10 and ADAM9 contribute to leukocyte migration.
In this study we show that treatment of leukocytes with a pharmaceutical ADAM10 inhibitor reduced both the in vitro transendothelial migration and the chemotaxis of the monocytic cell line THP1. The combined pharmaceutical inhibition of both ADAM10 and ADAM17 did not further suppress leukocyte migration in either experimental setting. The subsequent analysis of THP1 cells with a gene silencing of ADAM8, 9, 10 or 17 in transendothelial migration and chemotaxis experiments revealed that ADAM8 and ADAM10 are critically involved in THP1 cell migration. While ADAM9 did not seem to be required ADAM17 was only ofminor importance for the migration of THP1 cells.
This study analyses the role of the four proteases ADAM8, 9, 10 and 17 in the migration of leukocytic cells. It shows that ADAM8 and ADAM10 are crucial for the in vitro transendothelial migration and chemotaxis of the monocytic cell line THP1

Poster Session - Board 024 / 119

The role of macrophage-expressed IL-37 in atherosclerosis

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Background: Atherosclerosis is a slow progressive disease that is increasingly recognized to be a chronic inflammatory disease. The most prominent cell type in the pathogenesis of atherosclerosis is thought to be the macrophage, which are the first cells to infiltrate into the intima where they induce inflammatory events that further exacerbate the disease. Along with other investigators, we have found recently that the human protein IL-37, a relatively unknown member of the IL-1 family of cytokines with no known mouse homolog, has potent anti-inflammatory activity.
Objective: The objective of this study is to express IL-37 specifically in macrophages and determine the ability of IL-37 to reduce the production of pro-inflammatory cytokines and to regulate the accumulation of lipids in mouse bone marrow-derived macrophages (BMDMs).
Methods: These objectives are achieved using a macrophage-specific retroviral overexpression system utilizing the CD68 promoter to express IL-37 in mouse BMDMs via viral transduction of hematopoietic stem cells.
Results: Based on our promising preliminary data, we have found that IL-37 reduces the levels of pro-inflammatory cytokines and appears to play a role in macrophage lipid homeostasis in vitro.
Conclusion: IL-37 expression in mouse macrophages has an anti-inflammatory effect in vitro and also appears to regulate key cholesterol transport proteins, reducing cholesterol uptake in mouse BMDMs. This may prove to be atheroprotective in future in vivo studies using LDLR knockout mice to study the effect of IL-37 on plaque development and progression.

Poster Session - Board 050 / 120

Increased detection of Sin Nombre hantavirus RNA in antibody-positive deer mice from Montana, USA: Evidence of male bias in RNA detection

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Hantaviruses are widespread emergent zoonotic agents that cause unapparent or limited disease in their rodent hosts, yet cause acute, often fatal pulmonary or renal infections in humans. Previous laboratory experiments with rodent reservoir hosts indicate that hantaviruses are cleared from host blood early in the infection cycle, but can be sequestered long term in various host organs. Field studies of North American deer mice (Peromyscus maniculatus), the natural reservoir of Sin Nombre hantavirus, have shown that viral RNA can be transiently detected well past the early acute infection stage, but only in the minority of infected mice. Here, using a non-degenerate RT-PCR assay optimized for SNV strains known to circulate in Montana, USA, we show that viral RNA can be repeatedly detected on a monthly basis in up to 75% of antibody positive deer mice for periods up to 3-6 months. More importantly, our data show that antibody positive male deer mice are more than twice as likely to have detectable SNV RNA in their blood as antibody positive females, suggesting that SNV-infected male deer mice are more likely to shed virus and for longer periods of time. These results are the first non-behavioral data to help explain why the adult male deer mice are 3 times more likely to seroconvert within wild deer mouse populations.

Breakout Session IC: Infectious Diseases - Parasites & Vectors / 121

Reducing Angiostrongylus cantonensis infection on the Island of Hawaii through an integrated educational and research approach

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Background: The nematode Angiostrongylus cantonensis is a rat lungworm, a zoonotic pathogen that is considered a global, emerging infectious disease that causes rat lungworm disease (RLWD). Rats are the definitive hosts, slugs/snails are the obligatory intermediate hosts. Humans become infected by ingesting intermediate hosts containing infective third stage larvae, many times on fresh produce. The Island of Hawaii is the epicenter for RLWD in the U.S.A. While the Hawaii State DOH has reported only 38 cases of the disease since 2005, the actual number of cases is likely much greater due a lack of reliable diagnostic tests. While RLWD can be potentially fatal, relatively little is known about the host range and distribution of this parasite in Hawaii. RLWD is a preventable disease, however there is little information available to the public about how to best prevent RLW infection. Objectives: 1.To evaluate infection levels in multiple intermediate host species from the island of Hawaii to try to identify RLW 'hotspots'. 2.To conduct a study in Hawaiian rats to determine if RLW parasite DNA can be detected in host blood as an early diagnostic test. 3.To conduct a vaccine trial in rats to determine the efficacy of a vaccine against RLW. 4.To integrate RLWD education into the science curriculum for 2nd and 5th grade students to teach students and their families about RLWD prevention. Methods: Quantitative PCR will be used to quantify the levels of infection in intermediate hosts. We are collaborating with USDA-APHIS to complete two trials in rats to determine if qPCR can be used to detect RLW parasite DNA in blood, and evaluate the efficacy of a vaccine developed by collaborators in Spain. Our educational approach includes the development of RLW-based STEM curriculum appropriate for 2nd and 5th grade students. Results: We have developed a qPCR method for quantifying RLW parasites in intermediate hosts and are currently conducting an island-wide survey of intermediate hosts to determine host range and locate RLW 'hotspots' that can then be targeted for slug/snail control. As results become available, we are posting them at http://pharmacy.uhh.hawaii.edu/rlw/. The rat trials are currently underway. We have developed and are distributing an activity book entitled "The Mystery of Rat Lungworm Disease." The curriculum was piloted in 2nd grade classes at four schools in eastern Hawai'i in the fall of 2012. Presentations included interactive activities and demonstrations on how to clean and check produce and properly dispose of slugs and snails. Conclusion: These activities mark the beginning of a series of educational and research projects intended to limit the incidences of RLWD in the state and throughout the Pacific basin.

Poster Session - Board 035 / 122

The identification and characterization of a potential target of the F-box protein Cdc4p in Candida albicans.

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Background: Candida albicans is a major fungal pathogen of immunocompromised humans. C. albicans grows in yeast and filamentous/hyphal forms. Advances have been made in understanding the signaling pathways controlling the switch to filamentous growth, accompanied by changes in protein complement, and their role in virulence. However, the relationships between the signaling pathways, morphogenesis, and the cell cycle are not well understood. The SCF complex ubiquitin ligases and their subunits, such as Cdc4p are traditional cell cycle regulators that add ubiquitin peptides to "target" proteins. The F-box protein Cdc4p does not seem to play a critical role in cell cycle progression in C. albicans, but is a negative regulator of filamentous growth.

Cdc19p, a pyruvate kinase, is instrumental in glycolysis and ATP production. It was previously found at an increased level in cdc4/cdc4 C. albicans mutants, potentiating it as a Cdc4p target.

Objective: To determine if Cdc19p, a pyruvate kinase, is a target protein of the ubiquitin ligase complex.

Method: pCdc19 transformant cultures were prepared in an ampicillin rich environment following PCR, ligation, transformation, and digestion with restriction enzymes. The pGADT7/CDC19 construct will be utilized to transform Saccharomyces cerevisiae to assess interactions between Cdc19p and Cdc4p in a standard two-hybrid experiment. If the two proteins interact, the S. cerevisiae colony will successfully grow in an adenine depleted environment.

Results: Clones containing CDC19 and an A/T overhang vector were successfully developed. The next step will be to insert CDC19 into pGADT7 and then transform it into S. cerevisiae along with a plasmid containing CDC4.

Conclusions: We have successfully cloned CDC19 into the AT vector and are inserting it into the 2-hybrid system. Then we can determine if there is a positive Cdc19p/Cdc4p interaction. This, then, could indicate that pCdc19 is a direct target of Cdc4p, and may play a role in C. albicans morphogenesis.
The identification and characterization of a potential target of the F-box protein Cdc4p in Candida albicans.

Poster Session - Board 061 / 123

Ultrasound Targeted Microbubble Destruction and Nonviral DNA Vectors for Hepatic Gene Therapy

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Background: Ultrasound Targeted Microbubble Destruction (UTMD) is a platform technology that can deliver gene-expression vectors bound to lipid microbubbles to ultrasound-accessible organs through acoustic inertial cavitation. We are applying UTMD to the hepatic delivery of conventional (pcDNA3), piggyBac (pmGENIE) and Sleeping Beauty (SB100x) transposon-based, and minicircle (MC) DNA vectors with CMV and liver-specific promoters. We are evaluating reporter and the therapeutic human factor IX (hFIX) transgene expression in mammalian cells and mice. Human FIX is synthesized in the liver and deficiency or absence of this coagulation protein causes the disease Hemophilia B.

Objective: The aim of this research is to identify optimal nonviral vectors for noninvasive gene therapy and amelioration of the coagulopathy in Hemophilia B mice.

Methods: Reporter transgene expression was evaluated in HEK293 and HepG2 cells using luciferase assays and in the livers of UTMD-treated C57Bl/6 mice using bioluminescence imaging and histological assays. Nonrestrictive linear amplification mediated (nrLAM) PCR was used to evaluate genomic integration sites of the pmGENIE reporter vectors. Optimal vectors encoding the hFIX gene will be delivered to C57Bl/6 and FIX deficient mice by liver-targeted UTMD. FIX expression, hepatic localization, and toxicity will be assessed using ELISA, histological, and liver transaminase assays. hFIX activity will be measured by activated partial thromboplastin time clotting assays.

Results: A 10-fold increase in reporter expression was observed in HEK293 cells transfected with pmGENIE-luc over 3 weeks. In C57Bl/6 mice, we observed UTMD-mediated liver-specific expression of pmGENIE2-luc for an average of 24 days (n=12), compared to 4 days with pcDNA3-luc (n=7) (p=0.037). Reporter expression was initially located proximal to blood vessels but was more evenly dispersed throughout the liver past 3 days. Chromosomal integration sites were randomly distributed in genomic DNA samples from mouse 3T3 cells transfected with pmGENIE3-eGFP, but were targeted to specific chromosomes in livers from C57BL/6 mice transfected with pmGENIE3-luc (n=13), revealing an unexpected tropism for certain murine chromosomal sites in vivo. Robust transgene levels were initially observed from all vectors, in vivo, however, the intensity remained 10 to 1000-fold stronger in mice (n=4/group) from the pmGENIE3-luc and pZY53-luc treatments compared to pcDNA3-, SB100X-, or MC-luc over 2 weeks. The greatest hepatic specificity of transgene expression was observed for the alpha1 antitrypsin promoter-driven pZY53-luc, supporting the use of tissue-specific promoters to further enhance UTMD site-specificity.

Conclusion: The combination of UTMD and transposon-based expression constructs provides a minimally invasive strategy for delivery of therapeutic genes to the liver. This may be useful for the treatment of many hepatic gene deficiency disorders, including Hemophilia B.

Poster Session - Board 023 / 124

Cholesterol crystal production by endothelial cells and its impact on early atherosclerosis development.

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Atherosclerosis is an inflammatory disease that begins with a dysfunctional endothelial phenotype caused by endogenous factors such as blood lipid and cytokine/chemokine levels or exogenous factors like cigarette smoke and certain microorganisms. While performing experiments involving endothelial cells (EC) and lipoproteins we observed cholesterol crystal (CC) formation by the EC and decided to study if this phenomenon would contribute to early stages of atherogenesis. When EC were incubated with high but physiologic levels of LDL we observed CC formation within multivesicular bodies of EC and their subsequent transport and excretion to the basolateral side of EC. We then cultured human aortic endothelial cells on CC coatings and detected several pro-atherogenic changes in endothelial function such as increased permeability due to incomplete inter-endothelial junction formation, increased transmigration of mononuclear cells and decreased regeneration potential, indicating that the formation of subendothelial CC may be important for endothelial dysfunction in early atherogenesis. Interestingly, in ldlr-/- mice fed a high fat diet for only 1 week we could detect subendothelial CC deposition in the earliest stages of atherosclerosis. Our findings indicate the important role of EC in possibly creating the first “subendothelial space” through cholesterol metabolism and subsequent CC formation that may be critical for the very beginning of atherosclerotic plaque formation.

Breakout Session IB: Development & Reproductive Biology / 125

Co-expression of Tcf21 and PDGFRα is required for Leydig cell maintenance in the adult mouse testes.

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Background: Tcf21 is a basic helix loop helix (bHLH) transcription factor known to control cell fate in multiple cell populations. Tcf21 has essential roles in the development of the embryonic heart, lung, kidney, gonad and spleen. In the testes, the promoter region of Tcf21 is regulated by SRY, which initiates embryonic testes development. Platelet derived Growth Factor Receptor (PDGFRα) α is a receptor tyrosine kinase that also is expressed in and required for several tissue specific fibroblast populations. Tcf21 and PDGFRα show a high degree of overlap in interstitial cell populations of many organs including the kidney, lung, gonad, and heart. In the embryonic testes, PDGFRα is expressed in Leydig precursor cells, and has been shown to be a vital downstream factor of SRY mediated Leydig cell differentiation. In the adult testes, both Tcf21 and PDGFRα are expressed in Leydig cells. However, the precise molecular mechanism(s) regulating PDGFRα expression and Leydig cell differentiation/maintenance is currently unclear. Objective: In this study, we investigate the correlation of Tcf21 and PDGFRα expression in the adult testes and the role of PDGFRα expression in Leydig cell maintenance and regeneration. Methods: To do this, we took advantage of mouse strains utilizing Cre-Lox recombination technology to delete PDGFRα/β and induce red fluorescent reporter expression in Tcf21 expressing cells in a time specific manner. Additionally, mice endogenously expressing PDGFRα-GFP were induced to fluorescently report Tcf21 expression. Testes from these animals were isolated at specific time points after induction and subjected to staining for multiple markers, including Calretenin, phalloidin (actin) and smooth muscle actin. Additionally, FACS analysis was performed on cells isolated from the testes of PDGFRα-GFP mice that were either induced (and hence heterozygous for Tcf21) or uninduced (and hence WT for Tcf21). Results: Adult induction of Tcf21 reporter expression in PDGFRα-GFP animals resulted in distinct colocalization of PDGFRα and Tcf21 within the Leydig cell population. Ablation of PDGFRα/β resulted in distinct loss of Leydig cells and also decreased Tcf21 reporter expression. FACS analysis revealed increased PDGFRα expression in PDGFRα-GFP/Tcf21 heterozygous cells as compared to PDGFRα-GFP/Tcf21 WT cells. Conclusion: Co-expression of PDGFRα and Tcf21 is required for Leydig cell maintenance in the adult mouse testes. Expression of PDGFRα-GFP is dependent on Tcf21 expression and loss of PDGFRα/β results in reduced Leydig cells and diminished Tcf21 expression. These results underscore the importance of Tcf21 and PDGFRα expression in the adult mouse testes, particularly in regards to Leydig cell maintenance.

Poster Session - Board 027 / 126

Vascular Dependence on Cardiac Fibroblasts

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Background: Epicardial-derived cardiac fibroblasts have been suggested to play a major role in cardiac fibrosis, but their effect on the endothelium has yet to be characterized.

Objective: In this study, we aim to define the effect of specific cardiac fibroblast population loss on cardiac endothelium.

Methods: Mice with an inducible Cre recombinase under the Tcf21 promoter were crossed with mice containing one or two floxed Pdgfra alleles as well as a yellow fluorescent protein and/or tdTomato under the Rosa26 promoter. These mice were induced at various time points, and hearts were isolated and frozen embedded. Vessel diameter and abundance were measured using isolectin B4 and quantified.

Results: As expected, reporter-positive cell numbers decreased with the lineage-specific deletion of PDGFR alpha. Associated with these changes, the diameter of cardiac vessels increased, and abundance of blood vessels decreased.

Conclusion: Cardiac fibroblasts are a critical population within the heart, playing significant roles in extracellular matrix deposition and cell-cell communication with myocytes and fibroblasts. We show here that a population of cardiac fibroblasts communicate, directly or indirectly, with the cardiac endothelium. This communication regulates the number and diameter of blood vessels, and future studies will be focused to elucidate the factors required for this interaction.

Poster Session - Board 040 / 127

A map of the rainbow trout (Oncorhynchus mykiss)

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1. Background:
The mucosal surfaces of animals form important symbioses with commensal bacteria. This is one of the most ancient and conserved associations found in nature. Teleost fish have commensals living in association with their mucosal barriers. While the gut microbiome of fish has been characterized in the past, a geographical map of the fish microbiome is currently lacking.

2. Objective:
The aim of the present study is to fingerprint the microbiome of farmed rainbow trout (O. mykiss).

3. Methods:

Control rainbow trout were obtained from a local hatchery in New Mexico. Five sampling sites were investigated: anterior gut, posterior gut, gills, skin and nose. Total DNA was extracted and the 16s rDNA was amplified using barcoded primers (V1-V3 region). Amplicons were pyrosequenced using 454 Roche technology. Data was denoised in Amplicon noise and analyzed in Qiime. Alpha diversity, beta-diversity, principal component analysis and core microbiota analyses were performed.

4. Results:
Skin is the most diverse site in terms of its associated microbial community. The microbiome of the nose clusters together with the skin. The trout gills have a separate distinct microbial community compared to the other sites. The anterior and posterior guts have similar microbial communities, which are different from the other sites sampled.

5. Conclusion:
This work represents the first topographical characterization of the rainbow trout microbiome. Different mucosal barriers have distinct microbial communities associated with them, likely providing specific metabolic and immunological advantages to the host.

Poster Session - Board 012 / 128

aquaporin3b expression in Xenopus laevis in early gastrula stages

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Dr. Christa Merzdorf's Lab is studying developmental neurobiology in the model organism Xenopus laevis (African Clawed frogs). We are studying the very early developmental stages when the nervous system is just beginning to form. This process includes patterning the neural plate and neural tube closure, which are critical for central nervous system formation. If the neural tube does not develop correctly, it will result in birth defects. The purpose of my project is to determine where the gene aqp3b is expressed in gastrula embryos. When aqp3b was targeted by a morpholino injection during gastrula stages, defects in fibronectin matrix assembly were observed. This indicated the importance of aqp3b in gastrula stages and suggests a novel role for the gene in facilitating cell migration. An in situ hybridization method was used to both determine aqp3b expression during gastrulation and identify the extent of expression.

Breakout Session IB: Development & Reproductive Biology / 129

Immune regulation during pregnancy in a model marsupial, the gray short-tailed opossum Monodelphis domestica.

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Background
Metatherians, more commonly known as marsupials, are a distinct lineage of viviparous mammals that last shared a common ancestor with eutherians (placentals) at least 148 million years ago. Marsupials are distinct from eutherians in that they give birth to highly altricial young after a short gestation that is supported, in most species, by a choriovitelline type placenta. Whether or not there is regulation of maternal immune responses to paternal alloantigens during marsupial pregnancy has been a matter of debate. Indeed it has been suggested that lack of immune regulation during pregnancy may explain the evolution of short marsupial gestation times. Alternatively the maternal immune system may be unaware of the presence of the fetal allograft in marsupials due to lack of a highly invasive placenta.
Objective
To determine if the maternal immune system in a model marsupial, Monodelphis domestica, is being regulated during pregnancy for the purpose of fetal protection.
Methods
RNA was extracted from pregnant and non-pregnant opossum uterine tissue samples and used to make cDNA libraries which were used to generate high-throughput sequence data on the Illumina HiSeq 2000 platform. Illumina sequence reads were aligned to the M. domestica genome, quantified, and tested for differential expression using the "Tuxedo Suite" of bioinformatics tools (Tophat v2.0.6, Bowtie2 v2.0.5, Cufflinks v2.0.2).
Results
As in eutherians, pro-inflammatory cytokines such as IL-1, IL-6, and IL-17 are down-regulated during opossum pregnancy. Low levels of CD4 in all samples indicate presence of CD4+ helper T-cells in the uterus during pregnancy. There is little to no transcripts of T-box (T-bet) and fork-head box P3 (FoxP3) transcription factors, which are markers for Th1 and regulatory T-cells (Tregs) respectively. Since marsupials lack the FoxP3 promoter necessary to induce Tregs outside of the thymus, this result is not unexpected. There is low expression of Th2 cell marker GATA-binding protein 3 (GATA-3) in both pregnant and non-pregnant tissue. The presence of more Th2 than Th1 type T-cells indicates a bias towards antibody-mediated over cell-mediated immune responses and is consistent with observations of normal human pregnancy. The Th17 type T-cell marker, RAR-related orphan receptor gamma (RORC), is not down-regulated in pregnant samples. Since Th17 cells are one of the main producers of IL-17 this suggests that Th17 cells are being regulated during pregnancy in some way. However, unlike eutherians this is not due to decidual prolactin which is not produced in marsupials.
Conclusion
Overall the results support active regulation to inhibit inflammatory responses during pregnancy in the opossum, consistent with there being potential harm to embryos due to maternal immune mechanisms.

Poster Session - Board 077 / 130

The PyroSNP package: SNP discovery from Roche454 whole genome sequencing

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Background: Single nucleotide polymorphism (SNP) discovery is an important step in studies comparing the genetic composition of organisms or populations, yet it is not a straightforward process using Roche454 sequence data.

Objective: Our objective was to develop a SNP detection tool to simplify the discovery of SNPs from Roche454 data.

Methods: The PyroSNP package uses existing tools for sequence assembly, and parses this output. Each potential SNP is characterized using a straightforward method to assess the likelihood that each is an actual SNP and not an artifact of the sequencing process.

Results: Using the PyroSNP package, we were able to identify biologically relevant SNPs (verified by Sanger sequencing) in Toxoplasma Gondii.

Conclusion: The PyroSNP package makes SNP discovery from whole genome sequencing simpler and potentially more reliable.

Poster Session - Board 033 / 131

Impacts of Antigen Structure on the Geometric Properties of Antibody Aggregation

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Background: IgE antibodies bound to cell-surface receptors FcεRI crosslink via the binding of antigens on the cell surface. These aggregate formations stimulate mast cells and basophils and initiate the signaling cascade for cellular degranulation, resulting in an allergic response. Nearly 40% of the world's population suffer from allergies predicated by the formation of these aggregates. Experimental studies show that the spatial organization of IgE-FcεRI complexes in aggregates affect the transmembrane signaling that initiates an allergic response.

Objective: There are many factors that effect the size and shape of aggregates, such as the valency and conformational structure of the antigen. This structure controls the the number of receptors that can bind to a single ligand, e.g., the valency of the ligand and the types of aggregate structures that can form limited by steric hindrance. We are interested in finding the similarities that exist between aggregates made with different antigens since they initiate the same signaling cascade for cellular degranulation.

Methods: The geometric simulation of hundreds of antibodies and ligands aggregating is potentially computationally infeasible. In order to reduce this computational cost, we generate rigid body polygon-based models of large and complex molecular structure. These structures of reduced complexity are incorporated into a Monte Carlo-based simulation that provide 3-D details of aggregates. In particular, we simulate 2 antigen structures, a synthetic globular trivalent fibritin trimer (DF3) and a common shrimp allergen (PenA1) with 18 binding sites and a coiled coil structure. We simulate these antigen at a variety of concentrations.

Results: We show that we can capture a detailed look into the geometry of aggregation formation and compare our results to experimentally measured properties. We analyze such features as molecule bindings, aggregate size distributions and distances between molecules within an aggregate. We find differences in the shape and size of aggregates produced for the different antigens, but we do see similarities in the distances between receptor complexes in the resulting aggregate structures.

Conclusion: We demonstrate the utility of our methods on two antigen, one synthetic and the other real. We are able to see the differences in how the aggregates are constructed and similarities in the distances between receptors using these two very different antigen, supporting the idea that there exists a particular distance between receptor complexes that is necessary for cell signaling.

Poster Session - Board 006 / 132

Morphological Comparison of IKAP Deficient Mice Through Immunocytochemistry

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This study was done to phenotypically assess the Lefcort lab's "knock-out" mice which are a model for Familial Dysautonomia, or Riley Day syndrome. This disease affects the autonomic nervous system and has a variety of physiological and cognitive affects. The primary mode of investigation used was immunocytochemistry staining comparison of mutant to control mice. Conditional knock-outs were made where the gene was knocked out in either the entire central nervous systems or only in neural crest cells due to the fact that complete knock-outs died early in development. Different tissue types from the "knock-out" or mutant mice were examined including kidney, adrenal gland, brain, retina, optic nerve, and spinal cord which were each stained with specific primary antibodies. It was found that there is sympathetic innervation to kidneys and adrenal glands in mice with the neural crest knock-out showing evidence that the disease does not cause a problem with migration of neural crest cells. Neuronal cilia and optic nerve morphology was normal in the mutant mice as compared to the controls. Additionally, we developed a new procedure using antigen retrieval to demonstrate the lack of IKAP protein in the cells that were targeted for the Ikbkap deletion in the mutant mice.

Poster Session - Board 014 / 133

Positioning the Midbrain-Hindbrain Boundary (MHB) During Nervous System Development

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Background: I have been working for four months investigating the positioning of the midbrain-hindbrain boundary (MHB), which is an important step in embryonic neural development. In the Merzdorf lab, we study gene expressions and interactions at the MHB and how errors could occur to shift its position.
Objective: Using frog embryos, we study how the transcription factor Zic1 contributes to formation of the boundary, and what roles are played by cell division and apoptosis.
Methods: This is a new project for our lab; we are still learning how the staining techniques work and how to interpret results from these procedures. We perform two staining procedures. They are TUNEL staining which locates apoptotic cells, and pH3 staining which detects the dividing cells.
Results: I have been observing cells undergoing apoptosis and proliferation by performing TUNEL and pH3 staining procedures. I have also performed cryosectioning procedures to observe fine details of target cells. By the use of microscopy and photography, I have recorded my results. My preliminary results will be useful as controls for interpreting future experiments.
Conclusion: In the two years before I graduate, I will continue the project to observe the positioning of midbrain-hindbrain boundary. Then, by performing zic1 gene manipulations in the embryos, I will examine whether changes in zic1 expression lead to changes in the positioning of the MHB boundary.

Poster Session - Board 064 / 134

Boolean Network Model for Transcriptional Cell-cycle in Yeast

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Biological and chemical interactions within a cell are often represented as a network, since often a product of one reaction provides an input for other reactions. Many of the genes exhibit a switch-like behavior and one can approximate their behavior by Boolean switch models. In these models one assumes that the output of a gene is Boolean – the gene is either ON or OFF. Boolean networks have been studied for decades as reasonable models of regulatory systems. My project during this summer was to construct different Boolean network models for transcriptional cell-cycle oscillator in yeast. Using piece-wise linear equations allows us to analyze the dynamics of the wave pool network. We also studied expanded Boolean networks where gene can be in more than two states, which is consistent with a network element differentially affecting two or more elements. We propose multiple models and choose the best model. Insights gained from these models will allow us to propose new experiments for our experimental collaborators.

Poster Session - Board 083 / 135

IBEST Computational Resources Core

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Background: Researchers at the University of Idaho needed a computational resource in order to utilize current bioinformatics applications and conduct large-scale simulations to address a variety of questions relating to evolutionary biology.

Objective: Our objective was to build a high performance cluster environment and the support infrastructure needed to maintain and operate it during its lifetime.

Methods: Using NIH COBRE we leased Dell equipment to build the cluster and for purchasing support servers. The servers and cluster system are all driven using the open source Linux CentOS operating system. The cluster is controlled and scheduled using TORQUE and Moui. Using module environments researchers have access to a large list of bioinformatics and simulation programs. The backend storage system is driven by a parallel-distributed file system called Lustre that the cluster systems have direct access to.

Results: Researchers, both on and off campus, now have easy access to computational resources to run bioinformatics applications and run large-scale simulation that were previously unavailable at this scale.

Conclusion: Ibest's Computational Resources Core is able to provide personalized access to a subset of researches that need the computational resources of a cluster but do not need access to a super computer.

Poster Session - Board 007 / 136

Neural Correlates of Spatiotemporal Boundary Formation

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Purpose: In spatiotemporal boundary formation (SBF), surface boundaries, object shape, and global motion are perceived from sequences of local element changes (Shipley & Kellman, 1994a, 1994b). It has previously been suggested that SBF uses sequential changes as motion signals, and these feed into a computation that determines edges and eventually form (Shipley & Kellman, 1996). A model built on these assumptions is sufficient to model human performance in an edge discrimination test where the edges are defined by SBF (Erlikhman et al., 2012). However, no previous work has examined the neural correlates of SBF. Method: In a series of experiments, we applied EEG techniques to examine the neural timecourse of the formation of a shape representation in SBF displays. In one condition, four SBF shapes defined by color changes of small dot elements traveled on a circular path. In a control condition, the same number of changes occurred on every frame, but the sequence of the changes was randomized and no SBF shapes are perceived. In an initial study we collected EEG data recorded at electrode Oz. Results: The difference waveform elicited by subtracting the event related response to the control stimuli from that to the SBF stimuli consisted of a significant negative component from 200-300ms followed by a significant positive component from ~300-400ms. A control experiment determined that these differences likely represent the presence of global rotational motion. In a follow up study using a 256 channel HD EEG system, localized dipole sources corresponding to the formation of SBF contours were identified within both dorsal and ventral visual processing streams. Earlier dorsal sources likely reflect the extraction of the motion signals necessary for SBF (e.g., Kuba & Kubova, 1992) and later ventral sources likely represent the subsequent formation of the perceived shapes produced by SBF.

Poster Session - Board 087 / 137

TMCC Biomedical Pipeline Program

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Background
Truckee Meadows Community College (TMCC) is located in Reno, Nevada, has an enrollment of 12,868 students (four year average, 2009-2012), and is one of four outreach institutions within Nevada INBRE.

Objective
The goal of the TMCC Biomedical Pipeline Program is to increase the number of low-income, first-generation, and underrepresented undergraduates successfully working toward biomedical careers.

Methods
To reach our goals we have implemented a two-part workshop series. The Summer Bridge Workshop is an intensive five-week experience prior to freshman year where select students focus on math and language deficiencies, study skills, career awareness, and college preparedness. Students who successfully complete the Summer Bridge can apply to participate in the BioPrep Workshop the following summer. BioPrep is a hands-on laboratory experience designed to prepare students for success as life science majors by focusing on content knowledge in molecular biology, and essential laboratory and critical thinking skills.

Results
The 2012 Summer Bridge cohort included 191 incoming freshmen who were all first-generation college students, all low-income (i.e., Pell eligible), 54% female, 36% Hispanic, 12% multicultural, 3% African American, and 2% Native American. These students were recruited from local target high schools, 81% placed in pre-college math, 67% placed in pre-college English, and 53% were declared health or science majors. Students who completed the Summer Bridge Workshop in 2011 (n=225) had higher retention (71% vs. 66% enrollments with C or better in spring 2012) and higher fall-to-fall persistence (64% vs. 47%, fall 2011 to fall 2012) than a comparable low-income control group (n=788). Summer Bridge students were more similar to a non low-income control group (n=639, not Pell eligible) in retention (71% vs. 71%) and persistence (36% vs. 40%), and were enrolled in higher credit loads with 73% of Summer Bridge students attending at least 3/4 time (9-12+ credits) compared to 69% of the non-Pell and 67% of the Pell eligible control group. Between 2007 and 2012 a total of 51 students completed the BioPrep Workshop at TMCC, 78% were female, 61% Hispanic, 1% African American, and 1% Native American. Compared to a comparison group of 745 students who were Pell eligible and declared health or science majors, the BioPrep students had a higher rate of attaining an Associate's Degree (6% vs. 3%), higher transfer rates to a 4-year institution (29% vs. 15%), and a higher rate of attaining a Bachelor's degrees (14% vs. 1%) between Fall 2007 and Fall 2012.

Conclusion
The TMCC Biomedical Pipeline program is helping to increase the success of diverse students, which is the first step toward developing a diverse biomedical workforce.

Breakout Session IIB: Immunology & Tumor Suppression / 138

Knockdown of Osteoprotegerin reduces metastasis in breast cancer cells

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Background: Osteoprotegerin (OPG) is a secreted member of the tumor necrosis factor (TNF) receptor family that has been well characterized as a negative regulator of bone remodeling. OPG is also expressed in human breast cancer tissues and cells. In vitro studies suggest that OPG exerts tumor-promoting effects by binding to TNF-related apoptosis inducing ligand (TRAIL), thereby preventing induction of apoptosis. However, the in vivo effect of OPG expression by primary breast tumors has not been characterized. Analysis of OPG expression in human breast cancer cells lines shows higher expression in the basal as compared to luminal breast cancer subtype. Therefore OPG expression may be linked to more aggressive cell behavior including the ability to metastasize.

Objectives: The aim of the current study was to determine the impact of knocking down OPG in an in vivo model of breast cancer metastasis.

Methods: We knocked down OPG expression in MDA-MB-231 human breast cancer cells using shRNA and siRNA constructs to investigate impact on metastasis in the chick embryo model.

Results: We observed a reduction in metastasis with OPG knockdown cells. We found that the reduction in OPG expression did not alter sensitivity to TRAIL-induced apoptosis, but did observe reduced expression of the proteases Cathepsin D and Matrix Metalloproteinase-2 upon OPG knockdown.

Conclusions: We conclude that OPG has a metastasis-promoting effect in breast cancer cells and this may be mediated through modulation of protease expression. Further investigation into the value of OPG as a novel therapeutic target in breast cancer is required.

Breakout Session IIB: Immunology & Tumor Suppression / 139

The impact of conformational entropy on epitope selection by MHC class II

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Background and Objective. The adaptive immune response starts when CD4+ T cells recognize peptide antigens presented by class II molecules of the Major Histocompatibility Complex (MHCII). The interaction between peptides and MHCII is a thermodynamically cooperative process, characterized by the conformational flexibility of the reactants. However, the contribution of conformational entropy to epitope selection by MHCII needs to be elucidated.

Methods. Here, cycle-mutated peptides derived from influenza H3 HA306-319 are analyzed as they interact with the human MHCII allele HLA-DR1 (DR1) by isothermal titration calorimetry, fluorescence polarization, and circular dichroism (CD).

Results. Peptide ligation to DR1 displays enthalpy/entropy compensation that results in similar free energy of binding, even though individual enthalpy and entropy terms change significantly across the series. CD analysis indicates that complexes, whose entropic contribution to the binding is the greatest, feature spectra that are consistent with an incomplete conversion to the compact form. These distinct binding modes correlate with the stability of the complex during DM-mediated peptide release.

Conclusion. These results indicate that, at the molecular level, binding modes and thermodynamic binding signatures can be very different even for closely related peptides, and these signatures have a significant impact on the outcome of the epitope selection process.

Breakout Session IIB: Immunology & Tumor Suppression / 140

HSF1 Drives Resistance to Hsp90 Inhibitors by Promoting Autophagic Flux

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Background: Heat shock protein 90 (Hsp90) regulates the levels and activity of multiple client proteins including several oncogenes and tumor suppressors. Therefore, it contributes significantly to cancer progression and inhibitors of Hsp90 are in clinical development for both solid and hematologic malignancies. However, their efficacy is limited by the resulting activation of heat shock factor 1 (HSF1), as HSF1 directs the expression of genes that are cancer-promoting.
Objective: The use of Hsp90 inhibitors to treat cancer is limited by the fact that they elicit HSF1 activation. Our goal was to explore the mechanism by which HSF1 mediates resistance to the prototypic Hsp90 inhibitors geldanamycin and 17-allylamino-geldanamycin (17-AAG, tanespimycin).
Methods: Treatment of cancer cell lines with Hsp90 inhibitors shows clear activation HSF1 by luciferase reporter assay. We used the colorectal line RKO on account of robust HSF1 induction. To evaluate the role of HSF1 in drug resistance, we used siRNA and biochemical inhibitors. Calcein-AM was used to assess cellular viability in conjunction with Western blot analysis of apoptotic markers. Autophagic flux was evaluated by Western blot for LC3-II and p62 expression, as well as high content imaging of GFP-tagged LC3.
Results: Silencing HSF1 expression with siRNA, or blocking HSF1 activity with inhibitor KRIBB11 greatly enhances apoptotic cell death in geldanamycin and 17-AAG-treated cells. We further show that the mechanism by which HSF1 drives resistance to Hsp90 inhibitors is by promoting autophagy. To evaluate the link between HSF1 and autophagy, we monitored autophagosome biogenesis and autophagic flux in control and HSF1-silenced cells. In the absence of drug treatment, silencing HSF1 caused only a modest decrease in autophagosome numbers and autophagic flux, revealing that HSF1 is not critical for autophagy in normal, untreated cells. However, in the presence of geldanamycin or 17-AAG, HSF1 expression is essential for the maintenance of autophagy. Blocking autophagy with either 3-methyladenine or bafilomycin A1 increased apoptotic cell death following treatment with Hsp90 inhibitors, indicating a pro-survival role for autophagy in this setting.
Conclusions: These results highlight the potential of using HSF1 inhibitors as a unique strategy to interfere with autophagic flux and enhance the efficacy of Hsp90 inhibitors. Funding was provided by Hawaii IDeA Network for Biomedical Research Excellence (INBRE) project: NCRR Grant 5 P20 RR016467-10 and NIGMS Grant 8 P20 GM103466-11.

Poster Session - Board 008 / 141

When does frontoparietal neurostimulation benefit visual working memory in the healthy aging?

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Visual working memory (VWM) refers to the ability to maintain visual information over brief interruptions, such as eye movements. Cognitive decline, including a decline in VWM, accompanies the aging process. Because VWM plays a fundamental role in many aspects of cognition, there is great interest in developing interventions to postpone or counteract age-related cognitive decline. One approach involves the application of neurostimulation, such as transcranial direct current stimulation (tDCS). In our hands, we have found that tDCS to frontal and parietal regions in the healthy aging population can improve VWM function, but a series of experiments indicate that important caveats require further study. First, in several studies, our data show that benefits typically emerge when the VWM task is challenging. This is consistent with the notion of tDCS as a neuronal 'tipping factor' that may only make a visible difference when the cognitive demands are high and neuronal resources are exhausted. Second, group demographic differences such as education level appear to predict the degree of tDCS-related VWM benefit. Our data show greater improvements for those with more education. One possibility is that tDCS influences a strategic component of VWM tasks and that people with more education have more training and practice when selecting an appropriate strategy. Finally, longitudinal effects of tDCS-linked WM training suggest that tDCS may extend the benefits of training to untrained tasks. Furthermore, the timecourse of the emergence and disappearance of these benefits remains unclear. In summary, tDCS offers great potential for stabilizing VWM in general and special populations. However there are important factors that merit further study to best predict who will benefit from stimulation.

Breakout Session IIIC: Health Disparities / 142

Disparities and priorities for cancer in Nevada

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BACKGROUND
Little is known on the evidence-based priorities for research and public health interventions in the fight against cancer in Nevada.
OBJECTIVE
We used the most recent data available 2006-2009 from the Nevada Central Cancer Registry to carry out a thorough epidemiological assessment of cancer occurrence in the State.
METHODS
We compared cancer occurrence with the remaining states using the Surveillance Epidemiology and End results data from the National Cancer Institute and the Cancer Incidence in North America dataset. Data from Northern and Southern Nevada for screenable cancers (breast, colorectal and cervical) were used in survival models by using the Cox proportional hazards model, after adjustment for the most important confounders. The acute lymphoblastic leukemia cluster of 2000 was revisited, the downwinder cancer rates in areas to the east of the Nevada Test Site were studied and the out-of-state care load was estimated. Finally, racial-ethnic disparities on stage of diagnosis were assessed for the most common cancers, using logistic regression.
RESULTS
For all cancers combined, the standardized incidence rate ration for Nevada vs US was 0.95 for males (95%CI: 0.931-0.965) and 0.99 for females (95%CI: 0.984-1.023), but varied widely by cancer site. Nevadan women showed a substantially higher number of lung cancer cases (an excess of 202 per year) than expected. The out-of-state cancer care load was 9%.
For breast cancer, the most common cancer among females, Nevada is ranked 40 in incidence in the United States but occupies rank 5 in mortality, pointing to low survival. Survival rates after 4 years even after stratification for stage were consistently lower in southern Nevada (Las Vegas), compared to northern Nevada (Reno) and the US average. These differences persisted in the multivariate models. The survival disadvantage of the state as a whole extends to most other cancer sites. The uninsured and those on Medicaid were the most affected by late stage cancer.
CONCLUSIONS
Overall, Nevada has an average cancer rate compared to the nation, but it is first in incidence and mortality in the Western United States.
Further study of the tobacco –related cancer occurrence in Nevada women (both from tobacco smoking and second-hand smoke should be a priority), and a thorough assessment of the determinants for cancer survival disparities between Northern (Reno) and Southern Nevada (Las Vegas) are the main research priorities for the State.

Breakout Session IIIB: Cell & Molecular Biology / 143

Multimodal mechanism of action for the Cdc34 acidic loop: a case study for why ubiquitin conjugating enzymes have loops and tails

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Background: Mammalian cells contain some 600 ubiquitin ligases (E3s) and 40 ubiquitin conjugating enzymes (E2s), and yet E2s and E3s are highly selective when forming functional complexes with each other. The nature of how E2s and E3s establish specificity remains a largely unsolved problem in the ubiquitin field. Cdc34 is the principal E2 for the largest family of E3s, the cullin-RING ligases, and represents a good molecular system for uncovering the determinants of E2–E3 specificity.
Objective: Cdc34 consists of three molecular features: a canonical catalytic domain, a highly acidic C-terminal tail, and an acidic 12 residue insertion that is distal to the active site cysteine. While there are other E2s with either an acidic tail or an acidic loop, their combined presence is only found in Cdc34 orthologs and begs the following question: why does Cdc34 maintain these unique molecular add-ons? We and others recently determined that the C-terminal tail enables beyond diffusion rates of association with SCF, the archetypal cullin-RING ligase, which contributes to processive ubiquitination of Cdc34–SCF substrates. Much less is known about the acidic loop, although previous in vitro and in vivo experiments demonstrated that the loop is critical for Cdc34 function.
Methods: We use enzyme kinetics to characterize the specific effects of active site mutations on Cdc34 function. These results are followed up in vivo using yeast as a model system.
Results: Here we show that the acidic loop participates in multiple events during the Cdc34–SCF catalyzed ubiquitination reaction. First, the acidic loop increases the affinity of Cdc34 for SCF. Most significantly, we show that several conserved residues in the loop contribute to ubiquitin lysine deprotonation, an obligate step during the ubiquitination reaction.
Conclusion: Our work shows that both the C-terminal domain and the acidic loop are necessary for targeting Cdc34 activity to its cognate E3s, and furthermore that the presence of E3 stimulates the chemistry of isopeptide bond formation through a mechanism of action involving the acidic loop.

Poster Session - Board 038 / 144

Synthesis and evaluation of flavonoid and related phytochemicals as nature-inspired treatments for Clostridium difficile infection

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Background: Antibacterial flavonoids belong to a large family of polyphenol phytochemicals with a long history of use in ethnomedicine, but are largely unexamined against C. difficile.

Objective: Due to the increasing incidence, emergence of hypervirulent C. difficile strains, and the high rate of recurrence of Clostridium difficile infection (CDI), there is an urgent need for novel treatments for CDI.

Methods: A previously reported chemical library of flavonoid and flavonoid-like compounds was evaluated in this study. In addition, olympicin A and its inspired 4-chromanone and chalcone derivatives were designed and synthesized. Anti-difficile properties including minimum inhibitory and bactericidal concentrations, time-kill kinetic studies, effects on sporulation and toxin production, and membrane potential measurements were subsequently determined.

Results: With the exception of olympicin A, most naturally occurring phytochemicals tested were poorly active. Diversified synthetic flavonoids resembling olympicin A retained anti-difficile activity, suggesting olympicin A could act as a pharmacophore to obtain novel agents. They also demonstrated concentration dependent killing of logarithmic and stationary phase cultures and reduced sporulation and toxin production. In addition, olympicin A and some synthetic flavonoids dissipated the bacterial transmembrane potential.

Conclusion: Based on the potent anti-difficile properties of olympicin A and modified flavonoids, further exploration of this class of phytochemicals is warranted. These studies also demonstrate the potential for optimizing plant-derived flavonoids, and related antibacterial phytochemicals, as nature-inspired approaches to treat CDI.

Poster Session - Board 075 / 145

Assembly by Reduced Complexity

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Background:
Next Generation Sequencing (NGS) technologies produce millions of short reads from a set of longer template sequences. Assembly of these reads is a difficult problem which remains only partially solved. While most modern assembly programs have been designed for whole-genome assembly, this approach is not appropriate for some types of experiments, and can result in assemblies which are difficult to interpret and take large amounts of CPU time and memory to complete. Additionally, most assemblers use a variety of heuristics to speed up the assembly process. Because of this, assemblies are often lower in quality, and more fragmented when a large number of template sequences are represented in the pool of sequenced reads.
Objective:
We set out to design an automated pipeline we call Assembly by Reduced Complexity (ARC). The objectives for ARC include 1) break large, complex problems into smaller, more manageable ones; 2) reduce the memory footprint and CPU requirements associated with large assembly projects; 3) be highly scalable and take advantage of modern multi-CPU processors; 4) be easy to use, portable, and simple to configure.
Methods:
ARC uses an iterative approach for assembling reads associated with a prespecified set of “targets”. The algorithm consists of three steps. 1) Reads are mapped against a set of targets; 2) reads are then split into subsets based on the mapping results, and 3) assemblies are carried out for each target. This processes is then iterated using the newly assembled set of reads as mapping targets for the next iteration. ARC is implemented in Python and supports the Bowtie2 and BLAT mappers as well as the Roche/Newbler and Spades assemblers.
Results:
We present a novel approach to parallelize the assembly of multiple templates given a set of “target” sequences representing the templates of interest and a mixed set of reads. This approach has been tested on a number of problems including assembly of mitochondrial and chloroplast genomes, exome capture data, viral genomes, and bacterial plasmids. Assemblies are completed more quickly, and with increased accuracy by only considering those reads which should assemble together and reference bias is reduced as compared to mapping based approaches. ARC is open source and available at https://github.com/ibest/ARC.

Poster Session - Board 074 / 146

Reductive Biotransformation of PBDE-99 in Staghorn Sculpin and Starry Flounder Derived Hepatic Microsomes

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The reductive capacity of hepatic enzymes derived from southeastern Alaska forage fish toward brominated flame retardants and the role this biotransformation process may serve in disruptions to thyroid homeostasis was investigated. Hepatic microsomal fractions derived from fish liver tissue were characterized and used in assays designed to measure debromination of the polybrominated diphenyl ethers (PBDEs) and relative concentrations of the debromination products were compared to the relative abundances of PBDEs of lower bromine content found in concurrent tissue surveys of the parent species. While the majority of PBDE production and use in the US and Europe has been either banned or severely restricted since 2004, their environmental persistence is made apparent by contemporary surveys showing detectable levels in abiotic and biotic matrices. Moreover, the distributions of PBDEs with varying degrees of bromination (i.e. congeners) that are most common to flame retardant formulations is infrequently mirrored in the congener distributions found in biological matrices Reports of PBDE levels in fish species show larger proportions of lower brominated congeners suggesting reductive biotransformation is a significant attenuation pathway. PBDE levels were measured in tissue from staghorn sculpin (Leptocottus armatus) and starry flounder (Platichthys stellatus) collected from a PBDE impacted estuary in Juenau, AK and exhibit a disproportionate amount of the lesser brominated congeners. Liver tissue was harvested from the same species collected from a pristine site in Juneau, AK, microsomal fractions isolated, characterized and used in assays with the pentabrominated congener BDE99. Survey, microsomal assay results and evidence linking biotransformation of BDE99 in fish species to the deiodinase enzyme will be presented.

Poster Session - Board 081 / 147

The Nevada Bioinformatics Core

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Background: The Nevada Bioinformatics Center at the University of Nevada, Reno is an INBRE-supported Core facility, making Bioinformatics tools and services available to all Nevada and INBRE researchers.

Objective: Our goal is to help you perform biologically meaningful, computationally solid, and statistically robust research in the life sciences.

Methods: The Nevada Bioinformatics Center provides computational support, (bio)statistical analysis, experimental design consult, web interface development, information technology guidance, data storage, and general bioinformatics training and resources to Nevada and INBRE researchers and students.

We specialize in the analysis of next-generation sequencing data, including de novo genome and transcriptome assembly, identification of differential gene expression by RNA-Seq, and SNP-variant identification. One of our current favorite projects is the development of novel techniques to group, cluster, and display whole-transcriptome datasets, such as clusters confidence measures and co-expression networks.

The Core's computational infrastructure is impressive: our sequence analysis system alone consists of 32 cores, 184GB of RAM, and 24TB of disk space. We have plenty (64TB) of storage for our next-gen clients—we run a cutting edge distributed file system (GlusterFS), accessible over the network, capable of scaling to several petabytes of data and handling thousands of clients. The Core is also a contributing member of the UNR Research Grid, a High Performance Computing cluster of over seven hundred processor cores and 1.8 terabytes of RAM, available to all our collaborators.

We also support clinical and translational research by maintaining a HIPAA-compliant environment for clinical research, analysis of SNP genotyping data for genotype-phenotype association studies, and just recently, the analysis of transcriptional differences in cohorts using whole-genome sequencing technologies. The Core has become a statewide resource for general biostatistical support, offering Nevada researchers statistical advice on meaningful statistical tests (e.g. ANOVA), sound experimental design, power studies, hypothesis testing, and survival analysis.

Results: Since 2007, our Core has fostered more than 400 collaborations state- and INBRE-wide, with over 57 publications in the basic and life sciences. We have provided multi-disciplinary support for 10 successful grant awards in the last four years.

Conclusion: Our Bioinformatics Core has a strong record of published research and successful grant applications. Let us help you obtain your research goals!

This abstract made possible by grants from the National Center for Research Resources (5P20RR016464-11) and the National Institute of General Medical Sciences (8 P20 GM103440-11) from the National Institutes of Health.

Poster Session - Board 025 / 148

Chitinase Inhibition Promotes Atherosclerosis in Hyperlipidemic Mice

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Background:

Human macrophage chitinase-1 (CHIT1) activity is elevated in atherosclerotic patient sera and is present in atherosclerotic plaques.

Objective:

Elucidate the role of CHIT1 in atheroslerosis.

Methods:

Investigated the effects of a chitinase inhibition, on macrophage function in vitro and on atherosclerotic development in vivo. A chitinase inhibitor (allosamidin) was used to treat RAW 264.7 cells and Apolipoprotein E-deficient hyperlipidemic mice on an atherogenic diet.

Results:

In vitro, allosamidin treatment upregulated monocyte chemoattractant protein 1 and tumor necrosis factor alpha expression, and increased activator protein 1 and nuclear factor-kB transcriptional activity. Allosamidin decreased scavenger receptor AI, CD36, ABCA1, and ABCG1 expression. Apolipoprotein E-deficient hyperlipidemic mice treated for 6 weeks with constant administration of allosamidin and fed an atherogenic diet showed aggravated atherosclerotic lesion formation.

Conclusion:

These data suggest that CHIT1 exerts protective effects against atherosclerosis by suppressing inflammatory responses and polarizing macrophages toward an M2 phenotype, and promoting lipid uptake and cholesterol efflux in
macrophages.

Poster Session - Board 009 / 149

Mechanism of Inhibition of Recombinant GABAA Receptors by Pentylenetetrazole and Potentiation by Anticonvulsants

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Despite the wide prescription of drugs targeting postsynaptic γ-aminobutyric acid receptors (GABAARs) for seizure and epilepsy, mechanisms by which those drugs exert their effects on GABAAR are poorly understood. This is mainly attributed to the unavailability of crystal structure of the receptor and the various assembly of 19 different subunits (α1- α6, β1-β3, γ1-γ3, δ, ε, π, θ, ρ1-ρ3). Here, we studied the effects of two anticonvulsants on the recombinant α1β3γ2 GABAAR using whole-cell current recordings combined with rapid kinetic techniques. Our results showed that pentylenetetrazole (PTZ), a compound used to artificially induce convulsive effect in animal models, is a mixed inhibitor of the receptor. Anticonvulsant ethosuximide (ES) and the major metabolite (α-methyl-α-pheny-succinimide, MPS) of another anticonvulsant methsuximide (MS) were found to block the inhibition of the receptor by PTZ, while MS itself doesn't. Those data suggest that blocking the inhibition of postsynaptic GABAAR response is one of the mechanisms of the anticonvulsant effect of ES and MS. T-type calcium channel was suggested to be one of the targets of ES.

Poster Session - Board 010 / 150

Mechanism of Inhibition of α3β4 nAChR by a Sesquiterpene Lactone

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Tobacco consumption continues to be a leading cause of preventable illness and death in the United States according to the National Cancer Institute. According to CDC, currently 90% of lung cancer in men and 80% of lung cancer in women are caused by smoking cigarettes. However, after decades of new drug development, the treatment of nicotine addiction is still largely ineffective.

This study is focused on the inhibition of a sesquiterpene lactone (C8, isolated from Veronia cinerea (VC)) on the neuronal nicotinic acetylcholine receptor, the first and major target of nicotine. The VC plant has been used in Thailand and many other countries for smoking cessation, however the mechanism of action is not known. Our study showed that C8 is a non-competitive inhibitor of the receptor. In addition, the results suggest that C8 is a "silent desensitizer" and binds to the receptor allosterically because the compound caused desensitization without activating the receptor. Studies have shown that "silent desensitizers" could be beneficial in smoking cessation drug development because they will possibly have fewer side effects than the current smoking cessation drugs (Buccafusco, 2009).

Poster Session - Board 080 / 151

IBEST Genomics Resource Core Facility

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The Genomics Resources Core of the Institute for Bioinformatics and Evolutionary Studies provides biomedical researchers access to the expertise in molecular biology methods and bioinformatics needed to acquire, analyze, and visualize data generated from high throughput genomics technologies. The core facility offers investigators next-generation sequencing with the Roche 454 FLX+ long read pyrosequencing technology and shorter sequence read technology Illumina MiSeq, Roche NimbleGen microarray services for gene expression, ChIP-Chip, CGH and methylation studies, single nucleotide polymorphism (SNP) analysis for genotyping with Illumina BeadXpress, targeted re-sequencing to assess allelic diversity using the Fluidigm Access Array, and high-throughput sample preparation, quantitation and quality control. The core facility staff has expertise in both molecular biology and bioinformatics, enabling a holistic approach to genomics research that we refer to as the Interdisciplinary Triangle of Collaboration. Communication between all key personnel (principle investigator, molecular scientist and bioinformatician) begins early in study planning and design, continues through data generation, interpretation, and visualization, and doesn't end until manuscript publication. By doing so the IBEST Genomics Resources Core is able to contribute as full members of productive research partnerships.

Supported by grants from NCRR (5 P20 RR016448-10) and NIGMS (8 P20 GM103397-10) from the National Institutes of Health.

Poster Session - Board 011 / 152

Multimodal imaging of neuropsychiatric disorders (MIND)

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Background: A critical barrier to our understanding the neural mechanisms associated with psychosis and mood disorders is the inability to integrate multiple approaches (functional, structural and genetic) into a brain-based clinical assessment of neuropsychiatric disorders. Developing our knowledge of symptomatic similarities and differences that cut across diagnostic categories will allow a deeper understanding of our patients, and will promote more accurate diagnosis and treatment.

Objective: To improve the understanding the neural basis of psychotic and affective disorders will lead to improved diagnosis and treatment.

Methods: Four projects supported by an extensive clinical, biostatistics/neuroinformatics, and data analysis cores are focusing on distinct, but related, aspects of psychosis and mood disorders. Project 1 utilizes advanced data fusion methods to evaluate the ability of multimodal brain imaging data to differentiate patient groups and to push beyond discrete diagnostic categories by identifying individuals in intermediate positions on the continuum. Project 2 is an expansion of a program of genetic research involving the use of advanced multivariate methods to evaluate the shared and unique aspects of genetic influences on brain structural networks. Project 3 uses MEG and fMRI data fusion to determine neural network structure and dynamics underlying auditory verbal hallucinations (AVH). The initial aims are to isolate "core" AVH networks common to all patients and unique networks underlying differences in AVH phenomenology. The ultimate aim is to develop imaging-guided transcranial direct current stimulation as an AVH intervention. Project 4 uses a longitudinal design to study brain networks related to major depression and relapse after treatment with electroconvulsive therapy (ECT).

Results: We summarize the progress on each project to date. Project 1 has developed a multi-modal fusion method that successfully combines three imaging modalities to discriminate schizophrenia from healthy comparisons; the next step will be to include more clinical measures such as medication in classification and clustering. Project 2 has developed pipelines to process single nucleotide polymorphisms and copy number variant data and the methods to combine imaging data with genetics in patients with schizophrenia. Project 3 has identified AVH-related neural networks shared by fifteen participants in thalamic, lateral inferior prefrontal, and auditory cortex networks; the tDCS treatment protocol is now under development. Project 4 has identified differences in functional connectivity related to ECT response among subjects with a depressive episode; the next step will be to assess the pattern of changes in connectivity associated with a sustained ECT response in a larger data set of bipolar and unipolar depressed patients.

Conclusion: The projects are utilizing novel data analysis methods to identify both state and trait biomarkers associated with psychotic and affective disorders.

Poster Session - Board 037 / 153

Microwave-assisted organic synthesis of macrocyclic engelhardione as potential antituberculosis agent

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Background: Engelhardione is a natural product that originates from the roots of Engelhardia roxburghiana, part of the Juglandaceae family. Structurally, it belongs to a class of compounds called diarylheptanoids, which are found in many important medicinal molecules with antibacterial and anticancer properties. Engelhardione is a macrocyclic compound bearing a diphenyl ether scaffold linked by a linear 3-ketoheptane aliphatic chain. It was reported to show potent in vitro activity against Mycobacterium tuberculosis (MIC = 0.2 μg/mL).

Objective: Due to the emergence and spread of drug resistant Mycobacterium tuberculosis, there is an urgent need to discover new chemotype antitubercular agents with novel mechanisms of action.

Methods: Engelhardione was employed as a chemical starting point for subsequent structure-activity relationship (SAR) studies. The first total synthesis of engelhardione was very recently reported, and this effort led to the structural revision of this macrocyclic natural product. (L. Shen, D. Sun, Tetrahedron Lett., 2011, 52(35), 4570-4). The current work focused on the introduction of microwave-assisted organic synthesis (MAOS) in the macrocyclic step.

Results: engelhardione was synthesized through a series of aldol condensations and selective hydrogenation to construct the key linear building block, 1,7-diphenylheptan-3-one derivative, followed by the intramolecular macrocyclic Ullmann reaction and final demethylation.

Conclusion: This newly developed procedure toward macrocyclization proved to be highly efficient and afforded the macrocyclic product in 35 minutes in 85% yield.

Poster Session - Board 091 / 154

Aggregation and Interaction of Two Hormones that Regulate Food Intake

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Background: Ghrelin and obestatin, two appetite-regulating hormones, are produced in the stomach and likely stored as amyloid fibrils inside secretory granules.

Objective: Assess the propensity of ghrelin and obestatin to co-aggregate into amyloid fibrils, and the resulting impact these complexes have on monomeric hormone availability.

Methods: Ghrelin (acylated and desacylated) and obestatin (amidated and non-amidated) were studied in environmental conditions intended to mimic the pH of secretory granules (pH 5.5) and blood (pH 7.4). The formation of aggregates was measured over time using Thioflavin-T (ThT) dye fluorescence. Transmission Electron Microscopy (TEM) was used to visually verify the existence of fibrils.

Results: The ThT assay was negative for ghrelin samples prepared without obestatin. At pH 5.5 and pH 7.4, ThT fluorescence was observed in both obestatin and ghrelin-obestatin combination samples. TEM confirmed the presence of amyloid fibrils. 1H-NMR and LCMS are currently being used to identify the makeup of these fibrils. At pH 5.5, amidated-obestatin aggregated with itself to form fibrils much faster than non-amidated obestatin. At pH 7.4, both obestatin variants increased the rate at which they formed into amyloids.

Conclusion: Both amidated and non-amidated obestatin aggregate and form into amyloid fibrils, although at very different rates. Amidation appears to play a significant role in the comparatively rapid fibril formation observed at pH 5.5, a condition mimicking the storage environment of the secretory granules. At pH 7.4, both forms of obestatin increase the rate at which they form into fibrils.

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NIGMS Update

Sheila A. Caldwell, Ph.D., is a program director in the Division of Training, Workforce Development, and Diversity, where she manages IDeA Networks of Biomedical Research (INBRE) and Centers of Biomedical Research Excellence (COBRE) grants. In addition, she directs the Native American Research Centers for Health (NARCH) program. Caldwell was previously a program officer in the Division of Research Infrastructure at the former National Center for Research Resources. She earned a B.A. in international relations from Boston University and a Ph.D. in molecular and cellular oncology from George Washington University. Caldwell conducted postdoctoral research at the National Cancer Institute.

Welcome and NIH Updates / 156

Clinical and Translational Research Infrastructure Network (CTR-IN)

Over five years, 13 university partners will work as a “Clinical and Translational Research Infrastructure Network” (or CTR-IN), sharing resources and expertise designed to create new or better treatments that can be delivered in a rapid fashion from the laboratory to the bedside of patients.

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SBIR/STTP

The Small Business Innovation Research (SBIR) program is a highly competitive program that encourages domestic small businesses to engage in Federal Research/Research and Development (R/R) that has the potential for commercialization. Through a competitive awards-based program, SBIR enables small businesses to explore their technological potential and provides the incentive to profit from its commercialization. By including qualified small businesses in the nation's R arena, high-tech innovation is stimulated and the United States gains entrepreneurial spirit as it meets its specific research and development needs.

Poster Session - Board 093 / 158

A Collaborative Model of Biostatistical Support at UH JABSOM

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Clinical and translational research has become increasingly complex and multidisciplinary; such research relies heavily on scholars with expertise in research design and biostatistics. Historically, access to research design and biostatistical support for basic, clinical and translational research at University of Hawaii (UH) has been very limited, due to limited research design and biostatistical capacity, the fragmentation of related resources, and other fiscal and organizational barriers.

With the support of several NIH Institutional Infrastructural Grants, UH John A. Burns School of Medicine (JABSOM) has started the process of building a critical mass of biostatisticians and supporting staff. Assimilating with scattered and existing biostatistics resources across various fiscal and administrative barriers, the newly formed JABSOM Biostatistics & Data Management Core (BDMC) has grown into a nine-member dynamic and proactive group. Under a collaboration model, instead of individual investigator or program hiring grant-based biostatisticians, biostatistical personnel arrangement and funding can be coordinated ahead of time with the JABSOM BDMC, and adjusted based on the needs as the project/program progresses. A biomedical researcher can communicate with the biostatistics group at the earliest stage of a research project and in the development of a grant proposal. Collaboration through a structurally coordinated biostatistics academic “home” also provides broader biostatistics expertise coverage, increases efficiency and improves prioritization, streamlines collaborations and services, and allows biostatisticians to specialize in various research subject areas.

Since its establishment in 2011, the JABSOM BDMC has provided research design and data analysis support to over 300 projects, collaborated on 39 peer-reviewed publications and over 40 conference presentations. The group has supported over 60 grant applications and is currently supporting over $12 million grants annually. Besides biostatistical consultation and collaboration, the JABSOM BDMC is also actively involved in research design and biostatistical education and training, offered nice for-credit courses and 16 seminars or workshops during the period.

For more information, please visit: http://biostat.jabsom.hawaii.edu

The poster was supported by grants U54MD007584 and G12MD007601 from the National Institute on Minority Health and Health Disparities, and P20GM103466-12 from the National Institute of General Medical Sciences of the National Institutes of Health.

Poster Session - Board 092 / 159

Hawaii INBRE III, a Statewide Research & Education Partnership

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The Hawaii IDeA Network of Biomedical Research Excellence (INBRE), funded by NIGMS, is dedicated to the expansion of biomedical research capacity in Hawaii, with particular emphasize on natural products and pacific island health disparities. As Hawaii-INBRE III, we are focused on fostering and then nurturing research and education in biomedical science across the state at two critical junctures: undergraduate and early junior faculty. For undergraduates, this is achieved directly under our PATHway to Biomedical Careers program through our partners at our primarily undergraduate institutions (PUIs), as well as through the University of Hawaii, where student actively participate in laboratory research under a support student research experience (SRE). In addition, Hawaii-INBRE III supports newly developed independent research programs among our promising junior investigators at our PUIs, which, in turn, provide key sites for undergraduate research and education, in addition to those provided by our more established investigators. Strong mentoring programs are in place for our undergraduates and our junior investigators. A rich array of research and educational resources is provided through a large number of departmental and core facilities, including bioinformatics and biostatistics, genomics, proteomics, imaging, analytical biochemistry, synthetic chemistry, histopathology, antibody development, transgenics, cardiovascular phenotyping, behavior and electrophysiology. With highly diverse marine life and plant life in Hawaii as unique potential resources for natural products, along with our highly diverse population, there is considerable opportunity for discovery here in the biomedical sciences. INBRE III, together with our COBRE partners, is positioned to cultivate the next generation of biomedical scientists across our islands.

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Welcome

Poster Session - Board 094 / 161

Regulation of JNK activity by JIP1 scaffold protein mediates NMDA-dependent hippocampal learning and memory

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Background:
Although the c-Jun N-terminal kinase (JNK) signal transduction pathway has been implicated in neurodegenerative disorders, its role in learning and synaptic plasticity remains unclear. Our recent study provided the first evidence that the JNK pathway is critically involved in contextual fear conditioning under both stressful and baseline conditions. It has been implicated that the JIP1 scaffold protein may be required for JNK activation and it's effects on synaptic plasticity.
Objective:
To test if JIP1 is required for JNK activation and if this in turn mediates effects on learning and synaptic plasticity.
Methods:
We examined the phenotype of mice with mutation in the Jip1 gene (Thr-103-Ala) that selectively blocks JIP1-mediated JNK activation (JIP1TA mice). Behavioral paradigms were performed to test for hippocampal dependent spatial memory and contextual fear conditioning. Extracellular field recordings were measured to look for changes in LTP and further downstream biochemical analysis were performed to observe changes in sub cellular localization of NMDA receptor subunits.
Results:
We report that JIP1TA mice have enhanced hippocampus-dependent spatial memory, enhanced contextual fear conditioning, and lower threshold for hippocampal long-term potentiation induction. Changes in learning and synaptic plasticity in JIP1TA mice were attributed to a defect in JNK activation, and increase in total, surface and synaptic GluN2A and GluN2B subunit levels and enhanced signaling responses through NMDA receptors.
Conclusion:
Together, these observations demonstrate that JIP1 protein can influence hippocampus-dependent learning and synaptic plasticity through a regulation of expression and activity of NMDA receptors.