The 13th Annual DSAC Student Research Symposium
Thursday, January 14, 2016
Woodruff Health Sciences Center Administration Building (WHSCAB) Auditorium

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8:15 – 8:45 – Breakfast in WHSCAB Lobby

Session I: Nucleic Acids and Gene Expression ***8:45 am***

8:45 – Anna Knight (GMB)
DNA Methylation Predicts Neonatal Age at Birth

9:00 – Samuel Hong (MSP)
AP-1 Transcription Factors Bind Methylated Consensus Sequence

9:15 – Brindar Sandhu (GMB)
The Co-Evolution of Co-Dependent Enzymes

9:30 – Emily Rye Weikum (BCDB)
Untethering Glucocorticoid Receptor-Mediated Transcriptional Repression of Inflammatory Genes

9:45 – 10:00 – Break

Session II: Innovative Technologies ***10:00 am***

10:00 – Philip Zakas (MSP)
Bioengineering Coagulation Factor VIII through Ancestral Protein Reconstruction

10:15 – Robert Petit (PBEE)
Staphopia: A Web Application for Rapid Analysis of *Staphylococcus Aureus* Whole Genome Sequencing Projects

10:30 – Sara List (NS)
Representational Similarity Analysis Examining Bodypart fMRI Data

10:45 – Jessica Konen (CB)
A Novel Image-Guided Genomics Approach to Dissect the Mechanisms of Collective Cancer Cell Invasion

11:00 – David Nicholson (NS)
Simple, Accurate Method for Automated Classification of Birdsong Syllables

11:15 – 11:30 – Break

Session III: Translational Models *11:30 am*

11:30 – Lynette Chea (IMP)
Delaying Apoptosis Enhances Immunogenicity of MVA-Based Vaccinations

11:45 – Daniel Curry (NS)
Prosocial Effects and Neurotoxicity of (–)-3,4-Methylenedioxymethamphetamine in Mice

12:00 – Jessica McCaffery (IMP)
A Novel Simian Adenovirus Vector is Able to Elicit Humoral and Cellular Responses Protective Against an Experimental Plasmodium Challenge

12:15 – Fadi Pulous (CB)
Endothelial Cell Talin1 is Required for Postnatal Angiogenesis and the Maintenance of Established Blood Vessels

12:30 – 2:00 – Poster Sessions & Lunch in WHSCAB Lobby

Poster Sessions
12:30 – 1:15pm – Odd-Numbered Poster Presentations
1:15 – 2:00pm – Even-Numbered Poster Presentations

Session IV: Receptors and Signaling *2:00 pm*

2:00 – Kathryn Nawrocki (MMG)
CodY-Dependent Regulation of Sporulation in *Clostridium Difficile*

2:15 – Jarred Whitlock (BCDB)
Anoctamin 5: Implications in Muscular Dystrophy and Membrane Signaling

2:30 – MaKendra Umstead (CB)
Investigating the Role of Aurora Kinase A as a Positive Regulator of MAPK Signaling

2:45 – Andrew Swanson (NS)
Rho-Kinase Inhibition Augments Goal-Directed Decision-Making and Blocks Habitual Responding for Cocaine

3:00 – Lauren Byrd-Leotis (MMG)
The Use of Shotgun Glycomics to Identify Endogenous Receptors for Influenza Viruses in Natural Tissues

3:15 – 3:30 Break

Session V: Immunity and Infection *3:30 pm*

3:30 – Caitlin Bohannon (IMP)
Long-Lived IgM Plasma Cells Somatically Mutate in the Absence of Germinal Centers and Confer Long-Term Host Protection

3:45 – Nathan Jacobs (PBEE)
Indirect Competition Between Genetically Diverse Malaria Parasites is Mediated by Innate Immunity Independent of Virulence

4:00 – Kevin Sia (IMP)
CD40 Engagement is Required for Optimal IL-17 Induction During Tuberculosis

4:15 – Kathryn MacPherson (NS)
Modulation of Soluble TNF Signaling Alters CNS Immune Cell Populations and Rescues Impaired Synaptic Plasticity in 5xFAD Mice

4:30 – Sonia Laurie (IMP)
2B4 Signaling Regulates Metabolism in Alloreactive CD8+ T Cells Following Transplantation

4:45 – Lalita Priyamvada (MMG)
Evidence of Original Antigenic Sin in the Secondary Dengue Plasmablast Response

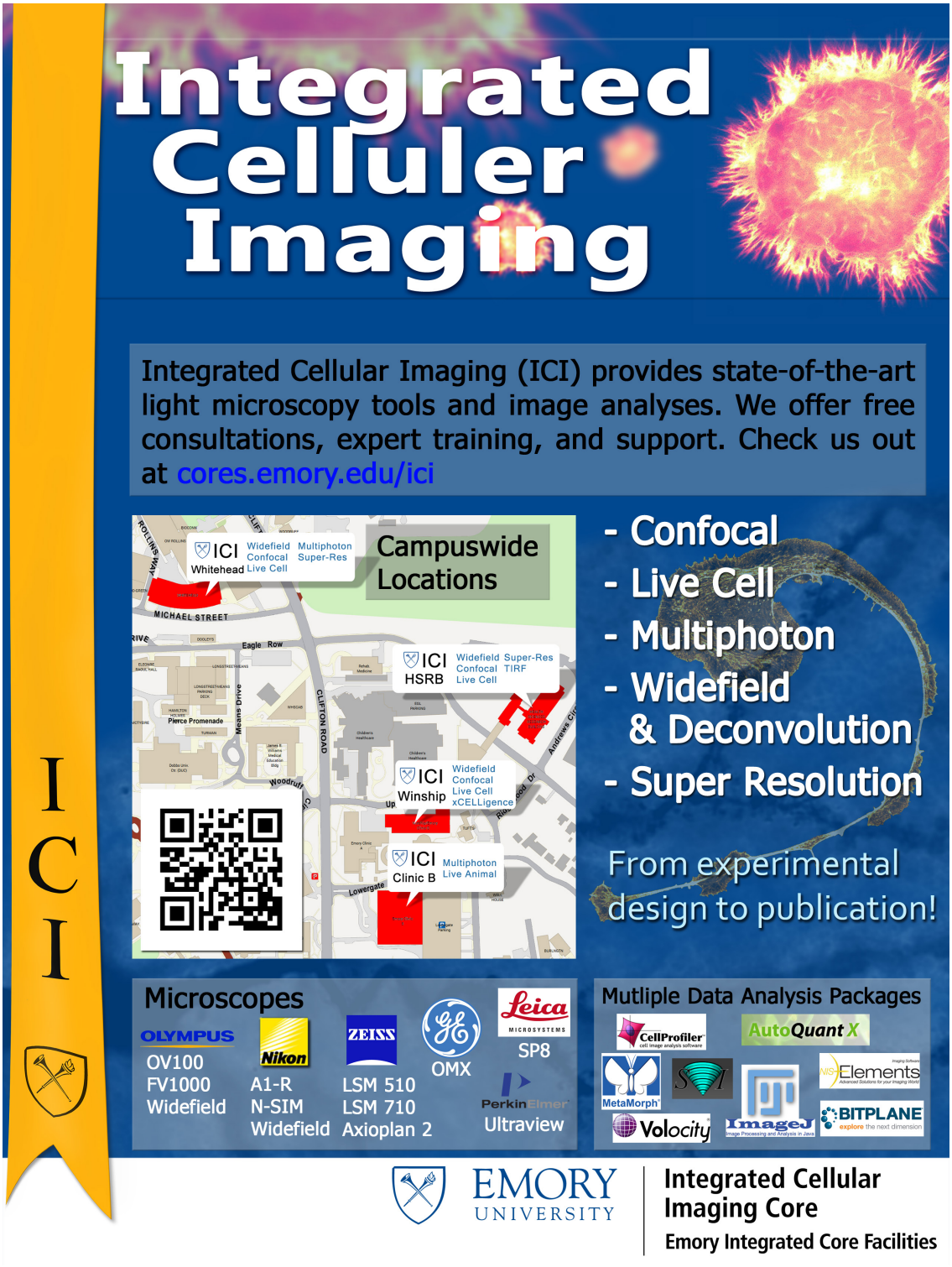
5:00 – 7:00
Reception and Award Presentations in WHSCAB Lobby

Images

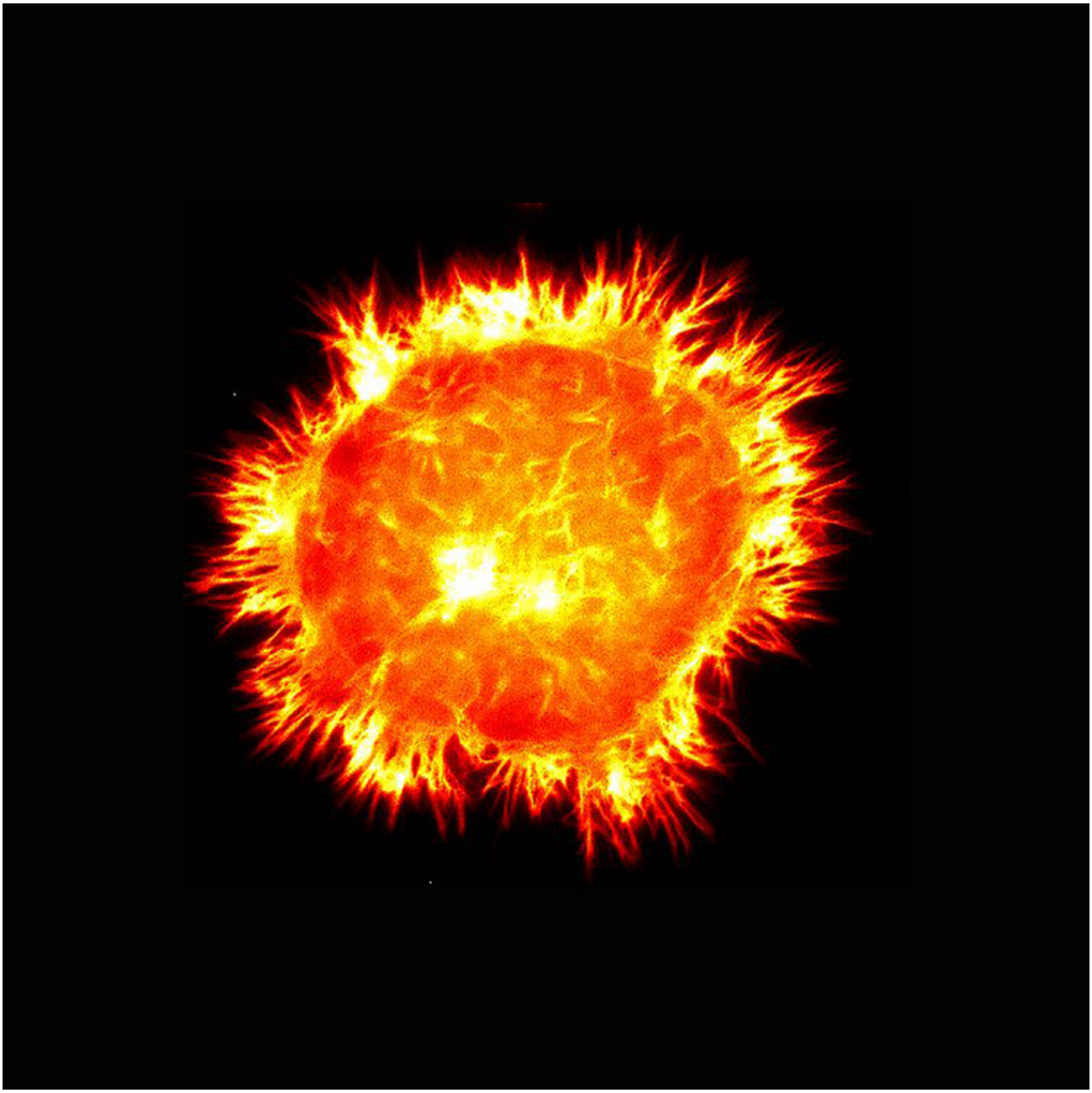
Image Contest Winners

1st Place: Stephanie Pollitt, NS – Page 5
2nd Place: Amanda York, BCDB – Page 24
3rd Place: Jadiel Wasson, BCDB – Page 62

All images were judged by the Integrated Cellular Imaging Center



Oral Presentation Abstracts



1st place – Stephanie Pollitt, NS

Actin cytoskeleton within a mouse neuroblastoma cell

Session I

Nucleic Acids and Gene Expression

8:45 am

Anna Knight, GMB **8:45 am**

DNA METHYLATION PREDICTS NEONATAL AGE AT BIRTH

Anna K. Knight[1]**,** Karen N. Conneely [1,2], Alicia K. Smith[1,3]
[1]Genetics and Molecular Biology Program, Emory University, Atlanta, GA, [2]Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, [3]Department of Psychiatry & Behavioral Sciences, Emory University School of Medicine, Atlanta, GA.

Accurate knowledge of gestational age (GA) is essential for proper monitoring and care of neonates. However, accurate GA measures are often not available. DNA methylation has previously been shown to associate with GA, and has been used to accurately predict chronological age in adults. We found that GA can be accurately predicted from DNA methylation of neonatal cord blood and blood spot samples (DNAm GA), using 148 CpG sites selected through elastic net regression in six training datasets (N=207). We evaluated predictive accuracy in six testing datasets (N=1,202), and found the accuracy of DNAm GA meets or exceeds accuracy of gestational age estimates based on established methods. We also found increased DNAm GA, relative to clinical GA, was associated with increased birthweight percentile (p=.00057), adjusting for covariates, suggesting DNAm GA could represent developmental age more accurately than clinical estimates of GA. Further development of this predictor could provide a method of accurate neonatal estimation of GA for use in resource-limited populations, or in cases where GA cannot be estimated clinically. When clinical estimates are available, the predictor can be used to test hypotheses related to developmental age and other early life circumstances, and may provide increased accuracy beyond clinical estimates.

Samuel Hong, MSP **9:00 am**

AP-1 TRANSCRIPTION FACTORS BIND METHYLATED CONSENSUS SEQUENCE

Samuel Hong[1,2] and Xiaodong Cheng[1].
[1]Department of Biochemistry, Emory University School of Medicine, 1510 Clifton Road, Atlanta, GA 30322
[2]Molecular and Systems Pharmacology Graduate Program, Emory University School of Medicine, 1510 Clifton Road, Atlanta, GA 30322

DNA methylation generally inhibits transcription factor binding. However, this notion has been challenged with recent identifications of several transcription factors that preferentially activate methylated promoters. AP-1 transcription factor, a master regulator of a wide range of cancer-related genes, has been shown to bind a novel consensus sequence in DNA methylation-dependent manner. Here, we present the X-ray structure of AP-1 in complex with an oligonucleotide containing the methylated consensus sequence. The structure reveals that a common motif conserved among bZIP proteins is involved in the specific recognition of methylated CpG as well as TpG. This finding accounts for two alternative sequence motifs bound by AP-1. Fluorescence polarization-based DNA binding assay reveals that AP-1 binds the methylated sequence several-fold better compared to the unmethylated version. Like 5mC, thymine (5-methyluracil) contains a methyl group at C5 position, and the similarity imposed by the methyl C5 preserves key structural features of DNA sequence recognized by AP-1. Our in vitro finding resonates with previous studies of AP-1-like viral transcription factor Zta, which preferentially binds methylated response elements. Our data suggest a novel way in which DNA methylation may function to activate transcription in a methylation-dependent manner.

Brindar Sandhu, GMB **9:15 am**

THE CO-EVOLUTION OF CO-DEPENDENT ENZYMES

Brindar Sandhu[1]
[1]Genetics & Molecular Biology

How do metabolic networks evolve? Such complex interactions can be reduced to protein complexes that have evolved from species to species. The bacterial enzyme γ-glutamyl kinase, or ProB, catalyzes the reaction that immediately precedes that of glutamate-5-semialdehyde dehydrogenase, or ProA, which takes place in L-proline biosynthesis. ProB and ProA act in a complex and ProB requires ProA for its function. I have shown that ProA-deficient *E. coli* cannot grow on minimal media, but that growth can be rescued by expression of *E. coli* proA on a plasmid. In order to test species differentiation in protein pathways, I have shown that expression of proA from the distantly related *Bacillus subtilis* and thermophile *Geobacillus stearothermophilus* do not rescue ProA-deficient *E. coli*. The inability of ProB-deficient *E. coli* cells to grow on minimal media suggests that ProB function is necessary for cell survival and that the distantly related ProA enzymes cannot interact with the *E. coli* ProB. The expression of both proB and proA from *B. subtilis* and *G. stearothermophilus* will determine if ProA and ProB have been forced to co-evolve due to their dependence upon interaction for survival.

Emily Rye Weikum, BCDB **9:30 am**

UNTETHERING GLUCOCORTICOID RECEPTOR-MEDIATED TRANSCRIPTIONAL REPRESSION OF INFLAMMATORY GENES

Emily Rye Weikum[1], William Hudson, Eric Ortlund[1]
[1]Department of Biochemistry, Emory University School of Medicine, 1510 Clifton Road, Atlanta, GA 30322

The glucocorticoid receptor (GR), a ligand regulated transcription factor that controls the expression of thousands of genes driving both up- and down-regulation at equal frequencies. The mechanism of gene activation is well understood and involves GR cooperatively dimerizing onto DNA at glucocorticoid response elements (GREs). However, the mechanism of repression is much more debated and is thought to occur though two possible mechanisms, DNA-dependent and DNA-independent. The DNA-dependent mechanism involves direct binding of GR to the newly discovered negative glucocorticoid response elements (nGREs). The DNA-independent mechanism involves GRs interaction with activator protein-1 (AP-1), which is a transcription factor in immune systems that drives expression of inflammatory response genes. This mechanism, known as "tethering", is the prevailing model for GR-mediated repression at inflammatory genes. However, recent discovery of GR-nGRE interactions lead us to wonder if DNA-dependent repression was possible at AP-1 response elements (TREs). We report that GR is able to repress inflammatory genes in the absence of AP-1, GR binds to TRE sequences *in vitro*, and crystal structures of GR bound to novel recognition sites located within canonical TRE-sequences. This data represents a paradigm shift in our understanding of GR-mediated repression and represents an alternative mechanism to the tethering hypothesis.

Session II

Innovative Technologies

10:00 am

Philip Zakas, MSP **10:00 am**

BIOENGINEERING COAGULATION FACTOR VIII THROUGH ANCESTRAL PROTEIN RECONSTRUCTION

Philip M Zakas[1], Kristopher Knight[2], Ernest T Parker[2], H. Trent Spencer[2], PhD, Eric Gaucher[3], PhD, and Christopher B Doering, PhD[2]
[1]Graduate Program in Molecular and Systems Pharmacology, Laney Graduate School, Emory University, Atlanta, GA
[2]Aflac Cancer and Blood Disorders Center, Emory University/Children's Healthcare of Atlanta, Atlanta, GA
[3] School of Biology, Georgia Institute of Technology, Atlanta, GA

The development of hemophilia A therapeutics has been hindered by the instability, immunogenicity and biosynthetic inefficiency of coagulation factor VIII (FVIII). Through the study of FVIII orthologs from existing species, we discovered unique molecular and biochemical properties that can overcome limitations of human FVIII. To expand this bioengineering approach, we employed ancestral protein reconstruction to generate a mammalian FVIII phylogenetic tree with predicted ancestral sequences. We resurrected 14 ancestral (An) FVIII sequences for molecular, biochemical and immunological characterization. Each An-FVIII displayed activity in standard coagulation assays using human plasma as a substrate, demonstrating evolutionary mammalian compatibility. To study biosynthetic efficiency, secreted FVIII and mRNA transcript levels were analyzed from stably transfected BHK-M cells. An-53, an ancestral sequence with 95% identity to modern human FVIII, displayed a biosynthetic efficiency 12-fold greater than human FVIII. Hemophilia A mice were also administered An-53 DNA within an AAV expression cassette via hydrodynamic injection. These mice displayed plasma FVIII levels 5.4-fold greater than the state-of-the-art human/porcine high-expression construct. Investigation of the ancestral sequences also revealed several FVIII molecules with increased stability following thrombin activation and/or reduced cross-reactivity to anti-human FVIII inhibitors, implicating other potential benefits in clinical settings.

Robert Petit, PBEE **10:15 am**

STAPHOPIA: A WEB APPLICATION FOR RAPID ANALYSIS OF *STAPHYLOCOCCUS AUREUS* WHOLE GENOME SEQUENCING PROJECTS

Robert A. Petit III[1] and Timothy D. Read[1]
[1]Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia, USA

As sequencing costs continue to decrease, whole-genome sequencing of bacterial pathogens directly from patients is becoming a common practice. Within the last few years, there has been a tremendous increase in the number of sequenced genomes publicly available from the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database. However, analysis and extraction of relevant information from these genomes to better understand the pathogen and the associated clinical phenotypes remains a challenge, as it requires extensive computational resources and bioinformatic skills. We have developed a proof of concept web-based platform called, Staphopia, for rapid analysis of large numbers of *Staphylococcus aureus* genomes. Publicly available *S. aureus* sequencing projects from the SRA database are processed through Staphopia's analysis pipeline. Raw sequence reads first undergo quality control filtration, normalization to a maximum of 50x coverage and *de novo* assembly. From the assembly, genes are predicted and annotated. Using sequence mapping, multi-locus sequence type (MLST) and variants (SNPs and InDels) are determined. *31*-mers are also counted for each project. To date, we have loaded more than 24,000 genomes from the SRA into Staphopia. Results of these analyses are stored within a database, which can be viewed at www.staphopia.com.

Sara List, NS **10:30 am**

REPRESENTATIONAL SIMILARITY ANALYSIS EXAMINING BODYPART fMRI DATA

Sara M. List[1], Simon Lacey[2], K. Sathian[2,3,4,5]
[1] Graduate Division of Biological and Biomedical Sciences, [2] Department of Neurology, [3] Rehabilitation Medicine, & [4] Psychology, Emory University, Atlanta, GA, USA & [5] Rehabilitation R&D Center of Excellence, Atlanta VAMC, Decatur, GA, USA.

A fundamental problem neuroscience faces is quantitatively linking brain-activity measurements, behavioral assays, and computational modeling. The purpose of this project was to implement a method to perform representational similarity analysis (RSA) on previously collected functional magnetic resonance imaging (fMRI) data. RSA is a form of multivariate pattern analysis which can be used to compare voxel-wise differences in activity across various conditions, allowing more in-depth information to be extracted from neural responses than can be seen from standard univariate activation differences. RSA can foster the comparison of response patterns for a set of stimuli through representational distance matrices, such that the distinction among stimuli that are functionally relevant can be determined. This method has proven to be an effective complement to standard univariate analysis. The data analyzed for the current project consists of blood oxygenation level dependent (BOLD) signals obtained when participants completed somatosensory, motor, and visual tasks for different body parts (e.g. face, arm, and leg). RSA and support vector machine learning (SVM) analyses both revealed somatosensory and motor areas share topographic mapping. Also, extrastriate body area (EBA) is sensitive to visual and motor stimulation. RSA is extremely flexible and can be used to compare fine-grained activity across conditions.

Jessica Konen, CB **10:45 am**

A NOVEL IMAGE-GUIDED GENOMICS APPROACH TO DISSECT THE MECHANISMS OF COLLECTIVE CANCER CELL INVASION

Jessica Konen[1,3], Bhakti Dwivedi[2], Jeanne Kowalski[2], Adam Marcus[3]
[1]Graduate Program in Cancer Biology, Emory University, Atlanta, GA, [2]Biostatistics and Bioinformatics, Emory University, Atlanta, GA, [3]Hematology and Medical Oncology, Emory University, Atlanta, GA

During tumor metastasis, cancer cells invade as a collective chain to navigate the microenvironment. This heterogeneous collective pack contains leader cells that pioneer invasion and follower cells that immediately follow the leaders. To dissect the mechanisms underlying this heterogeneity, we developed spatiotemporal genomic and cellular analysis (SaGA), a technique that precisely selects living cell populations within a physiologically relevant environment for downstream analyses. Thus we can select, isolate, and amplify any cell based upon predefined criteria. Using lung cancer spheroids, we precisely selected and extracted leader and follower cells from a 3-D matrix, thus creating the first leader and follower purified cultures. Leader cultures show dynamic invasive patterns, while follower cells show limited invasion. Reintroducing leader cells or conditioned media into follower cultures promotes their invasion and motility. Genomic analysis comparing leader versus follower cells shows enrichment in adhesion and VEGF signaling pathways in leaders. We confirmed that leaders secrete higher levels of VEGF compared to followers, and VEGF inhibition abolishes chain invasion. Overall, our data show that SaGA can select cells based upon dynamic behaviors and amplify subpopulations for downstream analyses. This method can impact the field of tumor heterogeneity by uncovering genomic signatures of subpopulations within cancer.

SIMPLE, ACCURATE METHOD FOR AUTOMATED CLASSIFICATION OF BIRDSONG SYLLABLES

David Nicholson[1]
[1] Neuroscience Program, Emory University

Songbirds provide a model system for the study of learned vocalizations. Each bird's song consists of repeated elements referred to as "syllables". To analyze song, experimenters label these elements by hand. However, songbirds produce thousands of songs a day, more than can be labeled. Some labs have developed automated analyses of birdsong, including various machine learning algorithms that classify syllables. One recent study used support vector machines (SVMs) to label Bengalese Finch song (Tachibana et al. 2014). Using their method, I find that the SVM method accuracy drops when faced with "introductory notes" (low-amplitude noisy syllables that often occur at the start of song). However I show that the simple K-nearest neighbors method yields greater than 99% accuracy on all syllables, including "introductory notes". I achieve this high accuracy by including features of preceding and following syllables in each sample of the training set. Studies in progress will determine how accuracy depends on the number of "neighbors" used to classify a syllable and the amount of labeled song in the training set. I will also determine whether using automatically labeled song affects typical results from the lab.

Session III

Translational Models

11:30 am

Lynette Chea, IMP **11:30am**

DELAYING APOPTOSIS ENHANCES IMMUNOGENICITY OF MVA-BASED VACCINATIONS

Lynette S. Chea[1], Linda S. Wyatt[2], Sailaja Gangadhara[1], Bernard Moss[2], Rama R. Amara[1]
[1]Emory Vaccine Center, Department of Microbiology and Immunology, and Yerkes National Primate Research Center, Emory University, Atlanta, Georgia, USA; [2]Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA

The development of an HIV vaccine to prevent and help control infection is critical to prevent the spread of HIV/AIDS. Modified vaccinia Ankara (MVA) is an immunogenic, attenuated poxvirus being developed as a viral vector for immunizations. However, MVA infected cells undergo rapid apoptosis leading to faster clearance of the antigen. To improve antigen persistence after MVA immunization, we introduced the anti-apoptotic gene, B13R, into the genome to enhance antigen-specific cellular immunity. The novel MVA-B13R construct protected infected cells from induced apoptosis better than wild-type (WT) MVA in an *in vitro* apoptosis protection assay. In C57Bl/6 mice immunized with recombinant WT MVA or MVA-B13R expressing SIV Gag Pol, similar kinetics and frequencies of Gag-specific $CD8^+$ T cells were observed. After a boost however, MVA-B13R immunized mice demonstrated enhanced Gag-specific $CD8^+$ T cell responses as detected in the tissues and periphery. Functional responses of $CD4^+$ and $CD8^+$ T cells against SIV Gag from both immunization groups were comparable, but MVA-B13R immunized mice demonstrated a trend for higher responses against certain regions of the SIV Gag sequence. These results demonstrate that delayed apoptosis of MVA-B13R infected cells quantitatively and qualitatively enhances the immunogenicity of MVA vaccines.

Daniel Curry, NS **11:45am**

PROSOCIAL EFFECTS AND NEUROTOXICITY OF (–)-3,4-METHYLENE-DIOXYMETHAMPHETAMINE IN MICE

Daniel Curry[1,2], Leonard Howell[1,3]
[1]Yerkes National Primate Research Center, Emory University, [2]Neuroscience Graduate Program, Emory University, [3]Psychiatry and Behavioral Sciences, Emory University School of Medicine.

(+/–)-3,4-methylenedioxymethamphetamine (MDMA) is an amphetamine derivative that became popular as a recreational drug (ecstasy) and therapeutic tool during the 1970's. Escalating use led to its prohibition but scientific interest in the drug has persisted due to its unique prosocial effects. Under clinical observation, volunteers report that MDMA increases feelings of closeness towards others, empathy, and gregariousness. In addition to these acute effects, there is evidence of enduring therapeutic effects such as improved interpersonal functioning and significant symptom reduction in PTSD patients. However, serious limitations remain to wider clinical use of MDMA, particularly its suspected neurotoxicity. There is thus significant impetus to isolate the prosocial mechanisms of MDMA from the neurotoxic effects. We investigated the hypothesis that (–)-MDMA may retain the prosocial effects of racemic MDMA but lack neurotoxicity. We found that both racemic MDMA (7.8 mg/kg) and (–)-MDMA (17 mg/kg) significantly increased murine social interaction. However, unlike racemic MDMA, (–)-MDMA did not induce hyperthermia or neuronal markers of toxicity such as gliosis or decreased brain dopamine content. These results indicate that the prosocial effects of MDMA are indeed separable from the neurotoxic effects, and that (–)-MDMA may be a more viable therapeutic than racemic MDMA.

Jessica McCaffery, IMP **12:00pm**

A NOVEL SIMIAN ADENOVIRUS VECTOR IS ABLE TO ELICIT HUMORAL AND CELLULAR RESPONSES PROTECTIVE AGAINST AN EXPERIMENTAL PLASMODIUM CHALLENGE

Jessica Nicole McCaffery[1]***, Jairo Andres Fonseca***[1], Monica Cabrera-Mora[1], Igor Dmitriev[2], David Curiel[2], Alberto Moreno[1,3].
[1]Emory Vaccine Center, Yerkes National Primate Research Center, Emory University, 954 Gatewood Road, Atlanta, GA 30329, [2]Cancer Biology Division, Department of Radiation Oncology, Washington University School of Medicine 660 S. Euclid Ave., 4511 Forest Park Blvd, St. Louis, MO 63108, [3]Division of Infectious Diseases, Department of Medicine, Emory University, 69 Jesse Hill, Jr. Drive, SE, Atlanta, GA 30303.

Malaria remains a considerable burden on public health. In 2015, the WHO estimates there were 214 million cases and nearly 438,000 deaths globally. A malaria vaccine is needed to reduce the burden of this disease. We have developed an experimental vaccine candidate (PyLPC/RMC) based on pre-erythrocytic (CSP) and erythrocytic (MSP-1) stage antigens derived from the rodent malaria parasite *P. yoelii*. Our protein-based vaccine construct is able to induce protective antibodies and CD4+ T cell responses. Based on evidence that viral vectors increase CD8+ T cell mediated immunity, we also have tested heterologous prime-boost immunization regimens that include human adenovirus 5 vector (Ad5). While Ad5 is commonly used for vaccine studies, the high prevalence of pre-existing immunity to Ad5 severely compromises its utility. Here, we report the use of the novel simian adenovirus 36 (SAd36) as candidate for a vectored malaria vaccine since this virus is not known to infect humans. Our studies show that SAd36PyLPC/RMC can enhance specific CD8+ T cell response and elicit similar antibody titers when compared to immunization regimens including Ad5PyLPC/RMC. The robust immune responses induced by SAd36PyLPC/RMC are translated into a lower parasite load following *P. yoelii* infectious challenge when compared to mice immunized with Ad5PyLPC/RMC.

Fadi Pulous, CB **12:15pm**

ENDOTHELIAL CELL TALIN1 IS REQUIRED FOR POSTNATAL ANGIOGENESIS AND THE MAINTENANCE OF ESTABLISHED BLOOD VESSELS

Fadi Pulous, Sara Fakhretaha Aval and Brian G. Petrich
Department of Pediatrics, Aflac Cancer and Blood Disorders Center, Emory University, Atlanta, GA, USA

Blood vessel development depends on integrin-dependent endothelial cell (EC) adhesion. Talin1 binding to β integrins functions to link integrins to the actin cytoskeleton and represents a key final step in integrin affinity modulation. To test the requirement of EC talin1 in postnatal angiogenesis, we administered tamoxifen to postnatal day 1 (P1) talin1(fl/fl),Cdh5CreERT2+ (talin1 EC-KO) and talin1(fl/fl),Cdh5CreERT2- (control) mice. Tamoxifen-treatment selectively deleted talin in ECs of talin1 EC-KO pups and led to death around P8 due to multi-organ vascular hypoplasia and hemorrhage. Analysis of retinal angiogenesis showed a 42% reduction in vascular radius and reduced blood vessel density in talin1 EC-KO mice. Empty matrix sleeves (collagen IV+/EC- structures) were more prevalent in talin1 EC-KO retinas suggesting that reduced vessel stability contributed to the reduced vascular density. To test the requirement of talin1 in the maintenance of established blood vessels, we treated adult (8-10 week old) mice with tamoxifen. Adult talin1 EC-KO mice died 16-20 days after tamoxifen treatment. Interestingly, gross pathology was most prominent in the intestines of talin1 EC-KO mice with hemorrhaging throughout the capillaries of the intestinal villi. Confocal microscopy of the intestinal vasculature revealed the rounding up of ECs cells and the formation of cyst-like structures comprised of several ECs. Together, these results indicate that EC talin plays an important role in postnatal angiogenesis and in the maintenance of established intestinal blood vessels.

Session IV

Receptors and Signaling

2:00 pm

Kathryn Nawrocki, MMG **2:00pm**

CODY-DEPENDENT REGULATION OF SPORULATION IN *CLOSTRIDIUM DIFFICILE*

Kathryn L. Nawrocki[1], Adrianne N. Edwards[1], Laurent Bouillaut[2] and Shonna M. McBride[1]*
[1]Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA, USA. [2] Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA, USA.

Clostridium difficile must form a spore to survive outside of the host. The factors that trigger sporulation in *C. difficile* are poorly understood. Previous studies suggest that a link exists between nutritional status and sporulation in *C. difficile*. We studied the impact of the global nutritional regulator, CodY, on sporulation in *C. difficile*. The role of CodY in sporulation was examined in the historical 012 ribotype and a current epidemic 027 ribotype. Sporulation frequencies were increased in both backgrounds demonstrating that CodY is a repressor of sporulation. The 027 *codY* mutant exhibited a greater increase in sporulation frequency than the 012 ribotype. The disparity between the effect of CodY on sporulation frequency between the 012 and 027 ribotypes demonstrates that the effects of CodY on sporulation can differ by strain. To determine the role of CodY in the observed sporulation phenotypes, we examined factors known to influence sporulation in *C. difficile*. Using transcriptional reporter fusions and qRT-PCR, we found that two loci associated with the initiation of sporulation, *opp* and *sinR*, are regulated by CodY. The data demonstrate that CodY is a repressor of sporulation and suggests that the impact of CodY on sporulation is influenced by the strain background.

Jarred Whitlock, BCDB **2:15pm**

ANOCTAMIN 5: IMPLICATIONS IN MUSCULAR DYSTROPHY AND MEMBRANE SIGNALING

***Jarred M. Whitlock*[1]**, Danielle Griffin[2], Louise Rodino-Kalplac[2] and H. Criss Hartzell[1]
1: Department of Cell Biology, Emory University, Atlanta GA.
2: Nationwide Children's Hospital, Ohio State University, Columbus OH

The importance of muscle mass and strength for daily activities such as locomotion and breathing is unequivocal. Deficits in muscle function produce muscular dystrophies that are characterized by muscle weakness and wasting and have serious impacts on quality of life and longevity. Recently, recessive mutations in TMEM16E (Anoctamin 5) have been directly linked to a variety of different muscle phenotypes including limb girdle muscular dystrophy type 2L and Miyoshi myopathy type 3, however the underlying pathogenic mechanism(s) has remained elusive. TMEM16E is a member of the Anoctamin/TMEM16 superfamily that encode both ion channels and regulators of membrane phospholipid scrambling. The phenotypic overlap of *TMEM16E*/ANO6 myopathies with dysferlin-associated muscular dystrophy has inspired the hypothesis that TMEM16E may be involved in muscle membrane repair and progenitor cell fusion to repair/regenerate muscle fiber lost or significantly damaged during injury. Here we characterize a novel murine *TMEM16E* knock-out murine model and investigate mechanistic underpinnings of the muscle phenotypes the mice manifest. Here we show that *TMEM16E*-deficient mice demonstrate muscle phenotypes reminiscent of limb girdle muscular dystrophy type 2L patients. We find muscle fibers isolated from these mice demonstrate a reduced capacity to repair the sacolemmela following laser damage, skeletal muscles display defective muscle regeneration following injury and we characterize a myoblast fusion defect impeding cell-cell fusion to produce multinucleated muscle fibers. We propose that these defects are caused by defective phospholipid scrambling normally elicited by TMEM16E at the plasma membrane.

MaKendra Umstead, CB **2:30pm**

INVESTIGATING THE ROLE OF AURORA KINASE A AS A POSITIVE REGULATOR OF MAPK SIGNALING

MaKendra Umstead[1,2], Jinglin Xiong[2], Cau Pham[2], Zenggang Li[2], Andrei Ivanov[2], Yuhong Du[2], Haian Fu[2,3]
[1]Cancer Biology Graduate Program, [2]Department of Pharmacology, [3]Winship Cancer Institute, Emory University

Aurora Kinase A (Aurora A), a protein known to facilitate mitosis, has emerged as a drug target for cancer therapy. Although Aurora A is amplified in several cancer types and increased expression is correlated to a worse prognosis for patients, the impact of increased Aurora A expression on cell signaling and cancer development remains unclear. One critical pathway through which mutations drive the development of cancer is the Ras-MAPK signaling cascade. When we co-expressed Aurora A and H-Ras in cells, we found that ERK phosphorylation increased compared to expression of Aurora A or H-Ras alone. Further, Aurora A requires the MAPK signaling cascade to potentiate ERK activation. Interestingly, we discovered that this effect correlates to a novel interaction between Aurora A and H-Ras. Through deletion analysis, the binding domains critical to mediate the interaction of Aurora A and H-Ras were characterized. We also determined that H-Ras is not an enzymatic substrate for Aurora A and the interaction may be kinase-independent.
As Aurora A gene expression is downstream of the Ras-MAPK signaling pathway, our data provide a mechanism by which Aurora A forms a positive feedback loop, revealing a role for Aurora A as a positive regulator of Ras-MAPK proliferative signaling.

Andrew Swanson, NS **2:45pm**

RHO-KINASE INHIBITION AUGMENTS GOAL-DIRECTED DECISION-MAKING AND BLOCKS HABITUAL RESPONDING FOR COCAINE

Andrew M. Swanson[1]**,** Lauren M. DePoy[1], Shannon L. Gourley[1]
[1] Yerkes National Primate Research Center, Emory University, Atlanta, GA

Both humans and rodents can learn to associate specific actions with their outcomes. With repeated performance, drugs, or stressor exposure, these actions can assume stimulus-elicited, or 'habitual,' qualities that are resistant to change. Several mechanisms have been identified by which decision-making strategies shift from action-outcome-based to stimulus-response-based habit systems, but *reversing* habits has proven difficult.
We isolated the role of Rho-kinase, a key regulator of the actin cytoskeleton, within the prelimbic cortex. First, we show that deep-layer prelimbic cortical dendritic spine density *predicts decision-making strategies*, such that higher densities are associated with stimulus-response habits, while lower densities are associated with engagement in action-outcome response strategies. Next, we show that fasudil, a Rho-kinase inhibitor, transiently reduces prelimbic cortical dendritic spine density and restores goal-directed decision-making in mice that have otherwise developed stimulus-response habits. Our findings further suggest that fasudil acts on the consolidation of new response-outcome conditioning. Finally, pairing fasudil with the devaluation of a cocaine reinforcer results in a marked delay in the acquisition of a new response for intravenous cocaine delivery. Together, these findings suggest that fasudil promotes action-outcome decision-making by augmenting the plasticity of deep-layer prelimbic cortical dendritic spines, and that it has therapeutic potential for cocaine abuse.

Lauren Byrd-Leotis, MMG **3:00pm**

THE USE OF SHOTGUN GLYCOMICS TO IDENTIFY ENDOGENOUS RECEPTORS FOR INFLUENZA VIRUSES IN NATURAL TISSUES

***Lauren Byrd-Leotis*[2], Renpeng Liu**[1,3], Konrad Bradley[2], Lasanajak[1,3], Sandra F. Cummings[2,3] , Xuezheng Song[1,3], Jamie Heimburg-Molinaro[1,3], Summer E. Galloway[2], Marie R. Culhane[4] ,David F. Smith[1,3], David A. Steinhauer[2], Richard D. Cummings[1,3]

[1]Department of Biochemistry, [2]Department of Microbiology and Immunology, and [3]The Glycomics Center, Emory University School of Medicine, Atlanta, GA, USA; [4]Minnesota Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN, USA

The hemagglutinin glycoprotein (HA) of influenza A virus is responsible for mediating virus attachment to host cells via glycan receptors terminating in sialic acid. The type of terminal sialic acid linkage confers species tropism, as avian and human strains preferentially bind ∝2,3-linked sialic and ∝2,6-linked sialic acids, respectively. To better define other characteristics of endogenous glycans that serve as viral receptors, we are exploring glycan recognition in tissues that are naturally infected by influenza such as the pig lung. For these studies, we utilized the novel technology of "shotgun glycomics" to identify natural glycan receptors. The total released N-glycans from pig lung glycoproteins were fluorescently-tagged, separated by HPLC, and printed on glass slides to generate pig lung shotgun glycan microarrays. We examined the binding of a panel of viruses representative of a range of HA subtypes and swine, avian, and human hosts. All viruses interacted with the sialylated N-glycans, and each virus demonstrated novel and unexpected differences in endogenous N-glycan recognition as compared to the structures bound on synthetic glycan microarrays. The results illustrate the repertoire of specific, endogenous N-glycans of pig lung glycoproteins for virus recognition, and offer a new direction for studying endogenous glycan functions in viral pathogenesis.

Session V

Immunity and Infection

3:30 pm

Caitlin Bohannon, IMP **3:30pm**

LONG-LIVED IGM PLASMA CELLS SOMATICALLY MUTATE IN THE ABSENCE OF GERMINAL CENTERS AND CONFER LONG-TERM HOST PROTECTION

Caitlin Bohannon[1]**,** Ryan Powers[1], Lakshmipriyadarshini Satyabhama[1], Ang Cui[2], Chris Tipton[1], Miri Michaeli[3], Robert Mittler[1], Ramit Mehr[3], Steven Kleinstein[2], Ignacio Sanz[1], and Joshy Jacob[1]
[1]Emory Vaccine Center, Emory University, [2]Yale University, and [3]Bar-Ilan University, Israel

Long-lived plasma cells are critical to humoral memory, continuously secreting neutralizing antibodies for as long as a lifetime. The long-lived plasma cells have previously been shown to be the result of antigen-stimulated B cells interacting with the germinal center, undergoing class-switching and somatic hypermutation. This results in long-lived IgG plasma cells that reside in the bone marrow. Here we show that antigen-specific IgM plasma cells can also persist in the spleen for a lifetime in response to a number of immunogens and pathogens. These IgM plasma cells can develop independent of the germinal center, but, interestingly, these cells are still able to somatically mutate via an AID-dependent mechanism. Additionally, we present data that antibodies from these IgM plasma cells can neutralize and protect against influenza viral challenge.

Nathan Jacobs, PBEE **3:45pm**

INDIRECT COMPETITION BETWEEN GENETICALLY DIVERSE MALARIA PARASITES IS MEDIATED BY INNATE IMMUNITY INDEPENDENT OF VIRULENCE

Nathan Jacobs[1], Jessie Barra[2], Rustom Antia[2], Tracey Lamb[3]
[1]Emory University Graduate Program in Population Biology, Ecology, and Evolution
[1]Emory University Department of Biology
[3]Emory University School of Medicine, Department of Pediatrics, Division of Infectious Diseases

In areas of intense malaria transmission, many individuals receive multiple infectious bites per night, leading to overlapping infections with multiple strains of *Plasmodium*. As the transmission likelihood of a given strain is influenced by blood-stage parasite densities, more competitively fit parasites are likely to be favored by evolution. Competitive ability has previously been linked to virulence, but mechanistic details are unclear. Here, we demonstrate a role for inflammatory monocytes in mediating competition between two clones of the rodent malaria parasite *Plasmodium chabaudi*. *Pc*AS and *Pc*CB grow to similar densities and cause similar degrees of anemia in separate single infections, but differ in the inflammatory response they elicit. In mixed infection, *Pc*AS densities were more adversely impacted than those of *Pc*CB, but competitive suppression was abrogated by depletion of monocytes prior to infection. Analysis by flow cytometry revealed that the monocyte population during mixed infection more closely resembles that of an infection with *Pc*CB than with *Pc*AS. Taken together, our results indicate that competition between genetically diverse malaria parasites can be mediated by innate immunity independent of parasite virulence, and that parasites gain a competitive advantage by polarizing the immune system towards a response to which they are adapted.

Kevin Sia, IMP **4:00pm**

CD40 ENGAGEMENT IS REQUIRED FOR OPTIMAL IL-17 INDUCTION DURING TUBERCULOSIS

Kevin Sia[1,2], Ranjna Madan-Lala[1], and Jyothi Rengarajan[1,3]
[1]Emory Vaccine Center, Emory University, Atlanta, GA, USA
[2]Immunology and Molecular Pathogenesis Graduate Program, Graduate Division of Biological and Biomedical Sciences, Emory University, Atlanta, GA, USA
[3]Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, GA, USA

Mycobacterium tuberculosis (Mtb) is a highly successful human pathogen that primarily resides in host phagocytes such as dendritic cells (DCs) and is controlled, but not eradicated, by CD4 T cells. IL-17 producing CD4 T cells (T_H17) play an important role in immunity to Mtb, but are poorly elicited during infection. Little is known about the molecular mechanisms generating T_H17s during infection or vaccination. We have previously demonstrated that Mtb impairs DC functions, including maturation, cytokine production, and antigen presentation. Since CD4 T cells are primed and polarized by DCs, we hypothesized that Mtb dampens T_H17 responses by modulating DC-T cell crosstalk. Here, we show that the costimulatory molecule CD40 on DCs plays an important role in polarizing T_H17 cells during Mtb infection. Mtb-infected CD40 knockout DCs poorly induce antigen specific IL-17 responses compared to wildtype DCs. The ability of CD40 to induce IL-17 responses depended on CD40-CD40L interaction between DCs and CD4 T cells. CD40 engagement utilizing trimerized CD40L boosted the ability of Mtb-infected DCs to induce antigen specific IL-17 *in vitro* and *in vivo*. Our studies highlight a role for CD40 in generating T_H17 and offer a potential avenue for improving the IL-17 response during Mtb infection.

Katherine MacPherson, NS **4:15pm**

MODULATION OF SOLUBLE TNF SIGNALING ALTERS CNS IMMUNE CELL POPULATIONS AND RESCUES IMPAIRED SYNAPTIC PLASTICITY IN 5XFAD MICE

MacPherson, K. P.[1], Sompol, P [2]. Kannarkat, G. T.[1], Chang, J.[1], Norris, C.[2], and Tansey, M. G.[1]
[1] Emory University School of Medicine, [2] University of Kentucky College of Medicine

Conditions of chronic peripheral inflammation, such as diabetes, and elevated tumor necrosis factor (TNF), are associated with increased risk for development of Alzheimer's disease (AD). Altered peripheral immune cell trafficking is evident in the brains of AD patients. TNF is known to have many functions including altering blood brain barrier (BBB) permeability and modulating glutamate receptor physiology. Soluble TNF (sTNF) induces proinflammatory signaling that plays a role in microglial activation and neurodegeneration. Systemic XPro1595, a biologic that selectively neutralizes sTNF without immunosuppression may ameliorate AD-like pathology induced by inflammation and/or regulate immune cell traffic across the BBB. In the 5xFAD transgenic mouse model we assessed changes in immune cell populations following peripheral dosing of XPro1595. We report that XPro1595 alters immune cell populations in both the myeloid and T cell compartments within the brain, as well as peripheral lymphoid organs. XPro1595 reduces microglial activation representative of a shift towards quiescent microglia. Brain slices from 5xFAD mice exhibited significant reductions in CA1 LTP, as compared to non-Tg mice, that was significantly increased with XPro1595 to near non-Tg levels. Experiments are ongoing to determine if these TNF-mediated changes in LTP and immune cell populations correlate with changes in amyloid burden and neurodegeneration.

Sonia Laurie, IMP **4:30pm**

2B4 SIGNALING REGULATES METABOLISM IN ALLOREACTIVE CD8+ T CELLS FOLLOWING TRANSPLANTATION

Sonia J. Laurie[1,2], D. Liu[1], and M. L. Ford[1],
[1]Emory Transplant Center, Department of Surgery, Emory University School of Medicine, Atlanta, GA 30322
[2] Graduate Program in Immunology and Molecular Pathogenesis

2B4 is an immunoglobulin superfamily member inducibly expressed on some CD8+ T cells. We recently showed in a murine skin transplant model that treatment with selective CD28 blockade resulted prolonged graft survival due to the upregulation of 2B4, which acted as a functionally important coinhibitor of T cell responses. We sought to determine whether the protective effect of increased 2B4 expression on graft survival is mediated by an impact on alloreactive T cell metabolism. We hypothesized that the loss of 2B4 would enhance the metabolic activity of graft-specific CD8+ T cells. To test this, we adoptively transferred 2B4-/- OVA-specific CD8+ OT-I T cells naïve animals, which then received an OVA-expressing skin graft. The loss of 2B4 resulted in rapid proliferation of donor-specific cells and enhanced expression of the fatty acid transporter CD36 following transplantation. 2B4-deficient OT-I cells expressed diminished levels of the glucose transporter GLUT-1 following transplantation, suggesting they may be less glycolytic than their wild type counterparts. Together, these data suggest that 2B4 signaling on CD8+ T cells may promote metabolic reprogramming, and raise the possibility that 2B4-mediated manipulation of cellular metabolism may be a novel strategy to target donor-reactive memory T cell responses that underlie rejection following transplantation.

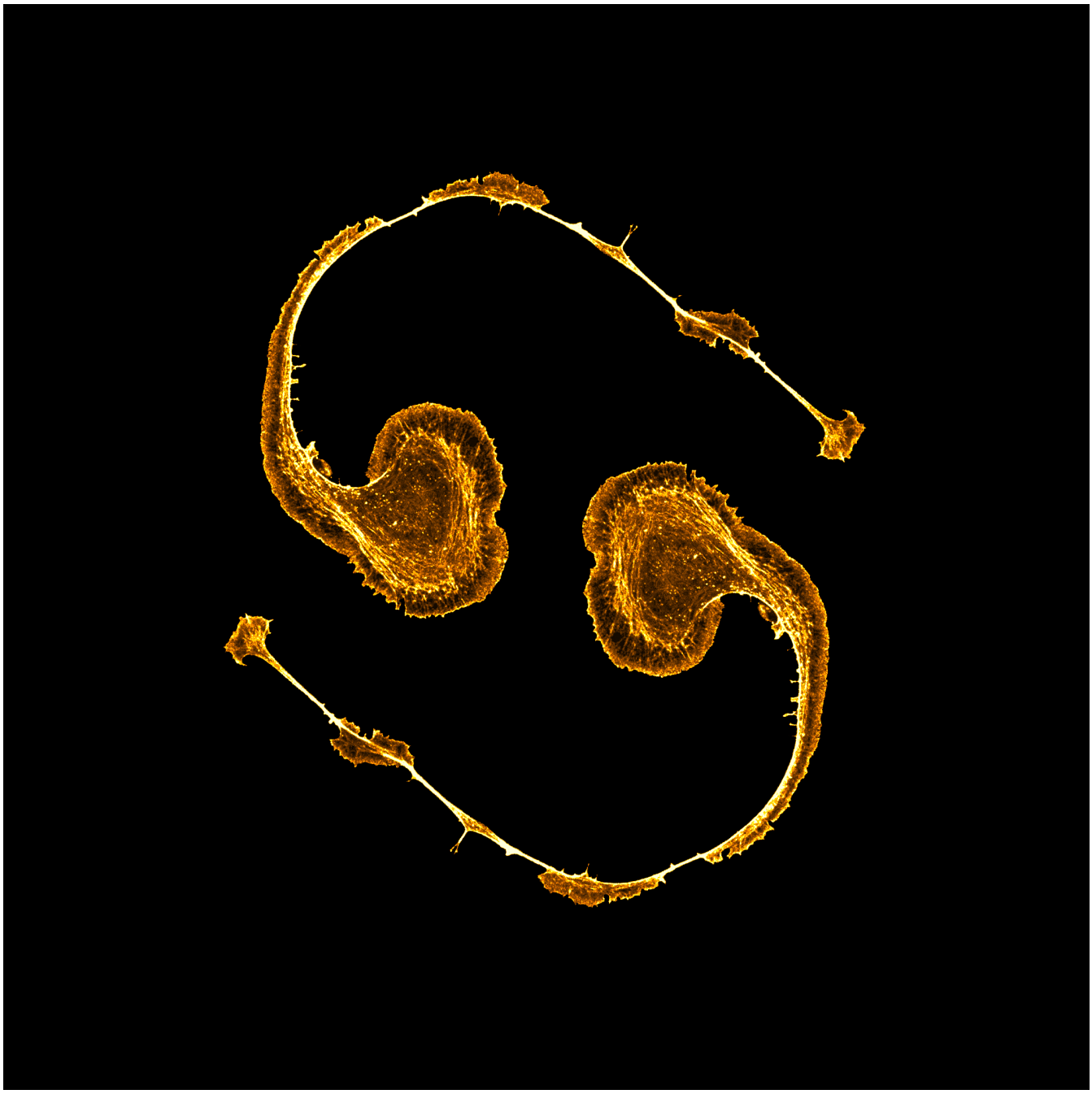
Lalita Priyamvada, MMG **4:45pm**

EVIDENCE OF ORIGINAL ANTIGENIC SIN IN THE SECONDARY DENGUE PLASMABLAST RESPONSE

Lalita Priyamvada[1], Alice Cho[1], Nattawat Onlamoon[2], Kulkanya Chokephaibulkit[2], Kovit Pattanapanyasat[2], Rafi Ahmed[1], Patrick Wilson[3], Jens Wrammert[1].
[1]Emory University, Atlanta, GA, United States, [2]Mahidol University, Bangkok, Thailand, [3]University of Chicago, Chicago, IL, United States.

There are four dengue virus (DENV) serotypes, and each can cause clinical disease ranging from undifferentiated fever to dengue hemorrhagic fever and dengue shock syndrome. Heterologous secondary dengue infections are associated with more severe symptoms, and original antigenic sin (OAS) is one of several factors hypothesized to contribute to this increased disease severity. In this study, we describe evidence of OAS in the acute phase B cell response of secondary dengue patients. We isolated plasmablasts from four Thai patients during ongoing infection and generated 53 monoclonal antibodies by single-cell immunoglobulin gene expression. The antibodies were largely cross-reactive to two or more DENV serotypes, with a small subset exhibiting serotype-specific binding and neutralization activities *in vitro*. Interestingly, although all patients were infected with DENV2 at the time of the study, a majority of the antibodies generated from two patients displayed stronger neutralization of DENV1 than DENV2. Despite DENV2 infection, the patients also generated antibodies that only neutralized DENV1 with low levels of cross-reactivity to DENV2 in binding. This neutralization bias towards a heterologous serotype is reminiscent of OAS, and could potentially attenuate protective responses against the current serotype of infection. Our studies provide basis for future work examining the impact of OAS-phenotype antibodies on protective immunity and disease severity in secondary dengue infections.

Poster Presentation Abstracts



2nd place – Amanda York, BCDB

Actin dynamics within a neuron

Poster Presenters

SESSION 1: 12:30 – 1:15PM -- Immunology & Molecular Pathogenesis (IMP), Microbiology & Molecular Genetics (MMG), Cancer Biology (CB), Molecular & Systems Pharmacology (MSP)
SESSION 2: 1:15 – 2:00PM -- Biochemistry, Cell & Developmental Biology (BCDB), Genetics & Molecular Biology (GMB), Neuroscience (NS), Population Biology, Ecology & Evolution (PBEE)

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2	Alicia Cutler	BCDB	38	Amy Dunn	NS
3	Allyson Koyen	CB	39	Madelyn Houser	IMP
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14	Edward Quach	BCDB	50	Jacob Billings	NS
15	Lauren Rusnak	CB	51	Elizabeth Littauer	MMG
16	Brittany Phillips	GMB	52	Julianne Freeman	NS
17	Scott Wilkinson	CB	53	Erica Bizzell	MMG
18	Kameryn Butler	GMB	54	Laura Butkovich	NS
19	Valentina Gonzalez-Pecchi	CB	55	Michele Daly	MMG
20	Crystal Grant	GMB	56	Maylen Perez Diaz	NS
21	Yun Wei	CB	57	Kyle Gerber	MSP
22	George Inglis	GMB	58	Olivia Moody	NS
23	Alice Cho	IMP	59	Lauren Shapiro	MSP
24	Christopher Rounds	GMB	60	Rachel Cliburn	NS
25	Ching-wen Chen	IMP	61	Katherine Squires	MSP
26	Marco Bajik	GMB	62	Natty Chalermpalanupap	NS
27	Christopher Lewis	IMP	63	Ju Young Kim	MSP
28	PamelaSara Head	GMB	64	Ashley Swain	NS
29	David Holthausen	IMP	65	Brandon Stauffer	MSP
30	Robert Haines	GMB	66	Joseph McMillan	PBEE
31	Emily Cartwright	IMP	67	Ayush Kishore	MSP
32	Sara Fielder	GMB	68	Dawn Barnes	BCDB
33	Emily Woods	MMG	69	James Cordova	MSP
34	Annie McPherson	GMB	71	Kristen Stout	MSP
35	Gordon Dale	IMP	73	Thayer King	IMP
36	Amielle Moreno	NS	75	Dana Tedesco	IMP

Alessandra Salgueiro, CB **Poster #1**

BEYOND EMT: THE ROLE OF VIMENTIN IN LUNG CANCER METASTASIS

Alessandra M. Salgueiro[1], Melissa Gilbert-Ross[1], Lauren S. Havel[1], John Shupe[1], Allyson E. Koyen[1], Hans E. Grossniklaus[1], Gabriel Sica[1], Adam I. Marcus[1]
[1]Emory University Winship Cancer Institute, Atlanta, GA 30322

Vimentin is a biomarker for metastatic potential and poor patient prognosis; however, little is known about how vimentin contributes to cancer progression. We created a novel genetically engineered mouse model (GEMM) to study vimentin in lung cancer metastasis. We first re-developed an LSL-$Kras^{G12D}$,$LKB1^{fl/fl}$ mouse. In this *Kras/Lkb1* GEMM (KLV+/+), approximately half the mice that develop primary lung tumors also develop metastasis. We used this model to test whether vimentin loss prevents metastasis by crossing this GEMM with a $Vim^{-/-}$ mouse to create a novel LSL-$Kras^{G12D}$, $LKB1^{fl/fl}$, $Vim^{-/-}$ (KLV-/-) mouse. We show that in the KLV-/- mouse primary tumor burden did not differ from KLV+/+ mice; however, KLV-/- mice exhibit significantly less metastasis compared to KLV+/+ mice. Histological analysis of primary tumors shows that KLV-/- mice exhibit less focal invasion than KLV+/+ mice. Vimentin staining of KLV+/+ mice was found in fibroblast-like cells surrounding invasive cell buds that we term collective invasion packs (CIPs). These vimentin-positive cells also stain positive for alpha smooth muscle actin, consistent with cancer-associated fibroblasts (CAFs). Taken together, these results suggest that in this background, vimentin may play a role in recruiting CAFs to invading cancer cells but not in cancer cell epithelial to mesenchymal transition (EMT).

Alicia Cutler, BCDB **Poster #2**

HIGH PURITY ISOLATION OF MYONUCLEI FROM SKELETAL MUSCLE

Alicia Cutler[1], Grace Pavlath[2]
[1]Biochemistry Cell and Developmental Biology, Emory University, [2] Muscular Dystrophy Association

While myofibers make up the bulk of skeletal muscle, the tissue is also comprised of many other cell types; including endothelial, immune, connective, and neuronal cells. When investigating nuclear processes in skeletal muscle one confronts two major obstacles: 1) nuclear proteins make up a small minority of proteins in the tissue due to the high abundance of cytoplasmic proteins, especially contractile proteins, and 2) both myonuclei and nuclei from other cell types will contribute to any assay performed. The obvious solution to both these difficulties is to selectively purify myonuclei from skeletal muscle tissue. However, pure myonuclei are challenging to isolate because skeletal muscle is full of structural components which are difficult to break up without damaging myonuclei and which co-sediment with nuclei, contaminating the nuclear fraction. We have optimized a technique by which intact myonuclei can be isolated with high purity from single mouse muscles. Purified nuclei are free of cytoplasmic and mitochondrial contamination. The nuclei are intact and suitable for use in a variety of downstream applications including flow cytometry, biochemistry, molecular biology, and proteomics. Proteomic analysis illustrates the increased depth of information available from isolated nuclei. This myonuclear isolation technique opens possibilities of examining changes in transcription, chromatin remodeling, and nuclear transport in aging, regeneration, and disease states without genetic myonuclear markers.

Allyson Koyen, CB **Poster #3**

A SYNTHETIC LETHAL SCREEN IDENTIFIES NOVEL DNA DAMAGE RESPONSE PROTEINS THAT MEDIATE CISPLATIN AND ETOPOSIDE RESISTANCE IN SMALL CELL LUNG CANCER

Allyson Koyen[1], Matthew Madden[1], David Yu MD, PhD[1]
[1]Department of Radiation Oncology, Winship Cancer Institute, Emory University, Atlanta GA

Small cell lung cancer (SCLC) is the most aggressive form of lung cancer, with a 6% five-year survival rate. Etoposide with platinum (EP) is the frontline combination chemotherapy for SCLC, however, many patients develop resistance and experience tumor recurrence. Etoposide and cisplatin both work by inducing DNA damage leading to cancer cell death. Cancer cells are known to rely on multiple mechanisms to maintain integrity of their DNA, and can develop compensatory mutations in DNA repair pathways that lead to chemotherapy resistance. Targeting critical junctures in DNA repair pathways may combat acquired chemotherapy resistance, however, DNA repair is complex many contributing players have yet to be identified. Here, we have conducted a siRNA screen in SCLC to identify genes that mediate resistance to cisplatin and/or etoposide, and have begun to characterize the strongest hits that have no prior link to DNA repair. Of particular note, CETN1, EZH2, and HELZ show regulated localization to sites of DNA damage, suggesting these may function directly in DNA repair. These novel DNA repair proteins show promise as targets or biomarkers for improving the efficacy of SCLC treatment.

Amanda Engstrom, BCDB **Poster #4**

LSD1 INHIBITION MAY CONTRIBUTE TO TAU-MEDIATED NEURO-DEGENERATION IN ALZHEIMER'S DISEASE

Amanda K. Engstrom[1]**,** Rohitha A. Moudgal[1]**,** Michael A. Christopher[1], Dexter Myrick[1], Benjamin G. Barwick[2], Allan I Levey[3], David J Katz[1]
[1]Dept. of Cell Biology, [2]Dept. of Microbiology and Immunology, [3]Dept. of Neurology, Emory University

Alzheimer's disease (AD) is an irreversible, progressive brain disorder caused by massive neuronal cell death in the frontal and temporal cortices and the hippocampus. AD is associated with the accumulation of β-amyloid plaques and neurofibrillary tangles of hyperphosphorylated Tau (NFTs). However, how NFTs contribute to neuronal cell death remains unclear. Recent data from our lab has demonstrated that the histone demethylase LSD1 is mislocalized with NFTs in AD cases. In addition, loss of LSD1 systemically in adult mice is sufficient to recapitulate many aspects of AD. These data raise the possibility that neurofibrillary tangles lead to neuronal cell death in AD by interfering with the continuous requirement for LSD1 to repress inappropriate transcription. Here we test this model by removing one copy of *Lsd1* from P301S Tau mice, which contain a human transgene overexpressing an aggregation-prone mutant of Tau. Interestingly, our preliminary results show these mice exhibit a faster and more severe neurodegeneration phenotype. This suggests that partial loss of LSD1 enhances the Tau-mediated neurodegeneration in this mammalian model. Our results indicate that Tau could be interfering with LSD1, and it may be possible to target LSD1 therapeutically to block the progression of AD.

Christina Ward, CB **Poster #5**

CHANGES IN TUMOR-ASSOCIATED MACROPHAGES FOLLOWING SYSTEMIC DELIVERY OF RECEPTOR-TARGETED NANOPARTICLES INTO MICE BEARING MOUSE PANCREATIC TUMORS

Christina Ward[1], Erica Bozeman[1], Weiping Qian[1], Y. Andrew Wang[2], Lily Yang[1]
[1]Winship Cancer Institute, Department of Surgery, Emory University School of Medicine, Atlanta, GA
[2]Ocean Nanotech, San Diego, CA

A hallmark of pancreatic cancer is the presence of extensive tumor stroma, such as tumor-associated macrophages (TAMs), that creates a physical barrier for drug delivery. Therefore, the development of therapeutic agents that are able to overcome this barrier would be significant for pancreatic cancer treatment. We developed urokinase plasminogen activator receptor (uPAR)-targeted magnetic iron oxide nanoparticles (IONP) using the mouse amino terminal fragment (mATF) of urokinase plasminogen activator (uPA) ligand with a polyethylene glycol (PEG) coating with or without Cisplatin (Cis) for targeted tumor imaging and therapy of pancreatic cancer. Systemic delivery of receptor-targeted IONPs in mice bearing Panc02 tumors led to accumulation of IONPs in tumors. CD68 (pan-macrophage) and CD163 (M2-like macrophage) double labeling showed a decrease in both macrophage populations in the tumor edge and center for the tumors treated with mATF-PEG-IONP with and without Cis compared to non-targeted and untreated controls. Prussian Blue and immunofluorescence double labeling showed uptake of receptor-targeted IONPs by CD68+ and CD163+ macrophages. This study suggests that targeted delivery into tumors could change the TAM burden in the pancreatic tumor microenvironment. This study could have clinical implications in IONP therapy agents for cancer detection and treatment.

Isaac Bishof, BCDB **Poster #6**

AN INTRINSICALLY DISORDERED LOW COMPLEXITY DOMAIN IS REQUIRED FOR U1-70K SELF-ASSOCIATION

Isaac J Bishof[1]**,** Ian Diner[1] and Nicholas T Seyfried[1].
[1]Department of Biochemistry, Emory University, Atlanta, Georgia, United States.

The RNA binding protein U1-70K aggregates via an unknown mechanism in Alzheimer's disease (AD). AD brain homogenates can induce the aggregation of soluble U1-70K rendering it detergent-insoluble. The intrinsically disordered C-terminus of U1-70K is necessary for aggregation and harbors two low-complexity (LC) domains, LC1 and LC2. Our objective is to determine if the LC domains of U1-70K are necessary for self-association. Addressing this question may provide mechanistic insight into the aggregation of U1-70K in AD. To investigate this question we performed co-immunoprecipitations of full-length rU1-70K and various mutants lacking either the LC1, LC2, or both LC domains. These assays revealed that the LC1 domain (amino acids 231-308) of rU1-70K is necessary and sufficient for association with native U1-70K. Similarly, we utilized bio-layer interferometry to measure the binding affinity of the LC1 domain with a synthetic peptide corresponding to a core region of the LC1 domain. These data provide evidence for a direct self-association between regions of the LC1 domain. Finally, by immunocytochemistry we show that rU1-70K mutants lacking the LC1 domain have an impaired ability to form nuclear granule structures. Together these data indicate that the disordered LC1 domain is necessary for U1-70K self-association and subnuclear compartmentalization.

Emily Summerbell, CB **Poster #7**

FARNESYLATED LKB1 ASSOCIATED WITH ACTIN TO PROMOTE STRESS FIBER FORMATION IN MESENCHYMAL CELLS

E. Summerbell[1,2], S. Wilkinson[1,2], A. Marcus[2]
[1]Graduate Program in Cancer Biology, Emory University, Atlanta, GA, [2]Hematology and Medical Oncology, Emory University, Atlanta, GA

The tumor suppressor LKB1 is a serine/threonine kinase and master regulator of cell polarity. LKB1 is the third most commonly mutated gene in non-small cell lung cancer (NSCLC). 72% of LKB1 mutations in NSCLC are truncations, resulting in loss of the C-terminal domain (CTD)-localized membrane-targeting farnesyl group; however, little is known about functional consequences of LKB1 farnesylation loss. Given that our lab has shown that LKB1 associates with actin at the leading edge of polarized cells and LKB1 farnesylation regulates cell polarity, we hypothesized that LKB1 CTD farnesylation regulates actin filaments to direct cell morphology during motility. Accordingly, we transfected LKB1-null HeLa cells with GFP-tagged LKB1: wildtype, farnesylation-motif mutation, CTD (lacking kinase activity), and farnesylation-mutant CTD. Cells expressing wildtype LKB1 or LKB1 CTD exhibit mesenchymal phenotypes and form distinct actin stress fibers, including lateral stress fibers and transverse arcs. Cells expressing wildtype LKB1 or LKB1 CTD exhibited strong leading edge LKB1-actin co-localization. Upon loss of LKB1 farnesylation, cells exhibit rounded amoeboid phenotypes, lack actin stress fibers, and lack LKB1-actin co-localization. We conclude that LKB1 CTD farnesylation induces formation of cell motility-related actin structures. Thus, LKB1 may regulate not only cell polarity, but also actin dynamics driving cancer invasion-related cellular morphologies.

Lindsey Knapp, BCDB **Poster #8**

ARMCX1, A POTENTIAL GENE FOR PLANAR CELL POLARITY REGULATION

Lindsey Knapp[1], Ping Chen[1]
[1]Department of Cell Biology, Emory University School of Medicine, Atlanta, GA

Planar cell polarity (PCP) is the coordinated polarization of cells in a tissue essential for embryonic development and organ morphogenesis. Membrane and membrane associated PCP proteins are partitioned asymmetrically at the plasma membrane to direct coordinated polarity among neighboring cells. These proteins send messages to cell machinery to direct intrinsic polarity. However, it is unclear how polarity signaling from the membrane is relayed to direct morphological polarity. A yeast two-hybrid identified Armcx1 as a protein interacting with the transmembrane core PCP protein, Vangl2. In situ hybridization confirmed Armcx1 is expressed in the mouse cochlea during the establishment of PCP. In the apical region of the sensory hair cells, Armcx1 is localized at the fonticulus, an actin-devoid region of the cuticular plate where the primary cilia, kinocilia, emerges, and medially at a previously unidentified distinct structure. The specific polarized localization of Armcx1 to the fonticulus and the unique medial structure suggests Armcx1 may play a unique role in PCP in the cochlea. Furthermore, Armcx1 appears to be associated with mitochondria in cells, implicating a potential involvement of mitochondria in PCP processes. Current studies focus on confirming the interaction between Armcx1 and Vangl2 and determining the subcellular localization of Armcx1 in cell culture and the inner ear.

Hye Rim Kim, CB **Poster #9**

GENOME-WIDE 5-HYDROXYMETHYLCYTOSINE ALTERATIONS IN MEDULLOBLASTOMA

Hye Rim Kim

Medulloblastoma (MB) is the most common malignant brain tumor in children and most of its morbidity results from relapse or metastasis. For many malignant tumors, genome-wide 5-hydroxymethylcytosine (5hmC) decrease has been recognized as one of hallmarks. Here we show the dramatic decrease of 5hmC abundance in MB, but with wide variations ($p<0.001$). Intriguingly, genome-wide 5hmC profiling indicated not only distinctive loss of 5hmC at the genomic loci observed in normal counterpart but the acquisition of 5hmC, particularly at the genomic loci implicated in the Notch signaling pathway. Furthermore, differentially hydroxymethylated genomic regions (DhMRs) in tumor tissues showed similar features with fetus, but not adult, suggesting that DhMRs in tumor prevent tumor from differentiation for normal brain development. By combining the 5hmC abundance and the postoperative survival of the patients, we found that the decrease of 5hmC could serve as a marker and unfavorable prognosis indicator of MB. These results imply a potential important role of 5hmC in MB.

Megan Allen, BCDB **Poster #10**

DIFFERENTIAL REGULATION OF CDK5 ACTIVATORS BY HUD CONTROLS NEURONAL CDK5 FUNCTION

***Megan Allen*[1],** Wenqi Li[1], Kevin Morris[2], Si'ana Coggins, Andrew Bankston, Wei Feng, Guanglu Liu[1], Yue Feng[1]
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The RNA-binding protein HuD plays key roles in neuronal development through post-transcriptional regulation of mRNA targets. Studies from model cell lines suggest that HuD stabilizes the mRNA that encodes p35, a protein activator for Cyclin-dependent kinase 5 (Cdk5) which phosphorylates substrates critical for brain function. Cdk5 activity, which is dysregulated in numerous neurological disorders, depends on the available amount of the distinct protein activators, p35 and p39. However, mechanisms for regulating Cdk5 activator expression in vivo have remained elusive. We show that HuD bind and stabilizes both p35 and p39 mRNAs in neurons. Surprisingly, elevated hippocampal HuD expression drives selective protein expression of p39 while p35 protein remains unchanged due to up-regulation of miRNA-101, a novel regulator of p35 expression. We observed increased p39-dependent Cdk5 activity in the hippocampus of transgenic mice harboring exogenous hippocampal HuD expression (HuDtg+). Furthermore, genetic removal of p39 ameliorates increased Cdk5 activity as well as the reported HuDtg+ phenotype of aberrant over-projection of mossy fiber axons from DGCs. In conclusion, our studies reveal a novel molecular mechanisms controlled by HuD which differentially affect expression of Cdk5 activators and a new functional link between HuD and the Cdk5 pathway in controlling neuronal circuitry in the hippocampus.

Jamie King, CB **Poster #11**

MITOTIC KINASE TTK AS A MODULATOR OF THE EPITHELIAL TO MESENCHYMAL TRANSITION IN BREAST CANCER.

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TTK (MPS1) kinase has established roles in DNA repair, centriole duplication and the spindle assembly checkpoint (SAC). In breast cancer, TTK is overexpressed at the protein level in triple negative (TN) and Her2+ tumors. Through early examination of TTK's effect on the proliferation of TN cell lines, we noted a morphological change suggestive of these cells reverting to an epithelial phenotype upon TTK silencing. We hypothesized that TTK overexpression may promote the epithelial to mesenchymal transition (EMT) in breast cancer cells. In patient data in cBioportal, TTK amplification is associated with increased vimentin but decreased E-cadherin gene products. In functional studies to date, we have observed that silencing TTK in TN breast cancer cells results in decreased protein and mRNA levels of mesenchymal marker vimentin, as well as increased levels of epithelial marker E-Cadherin. We have also observed blocking TTK kinase activity results in decreased vimentin protein levels. Future studies will determine mechanisms for how TTK modulates EMT, such as transcriptional regulation or protein-protein interactions. This work will provide novel information about this mitotic kinase's role in altering the behavior of breast cancer cells and provide a potential therapeutic target for TN cancers, which predominantly affect patients of African descent.

Omotola Omotade, BCDB **Poster #12**

POINTED-END CAPPING OF ACTIN FILAMENTS BY TROPOMODULIN REGULATES DENDRITIC SPINE DEVELOPMENT

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Dendritic spines are tiny, actin-rich protrusions on the surface of dendrites that serve as the platform for postsynaptic specializations of most excitatory synapses in the vertebrate central nervous system. Dynamic remodeling of the actin cytoskeleton drives the structural changes associated with the formation and modification of dendritic spines during development and synaptic plasticity, but the detailed mechanisms remain poorly understood. Tropomodulins (Tmods) are a conserved family of proteins that cap the pointed end of actin filaments, thereby regulating the stability, length, and architecture of actin networks in non-neuronal cell types. Genetic knockout of Tmod2 in mice leads to defects in learning and memory, but the underlying cellular mechanisms are unclear. In this study, we investigated the role of Tmods in dendritic spine development and synapse formation. shRNA-mediated knockdown of Tmods in cultured hippocampal neurons causes a marked loss in the number of mushroom-shaped dendritic spines, with a concomitant increase in filopodia-like protrusions. These results provide the first evidence that pointed end capping of actin filaments by Tmod plays a role in the development of dendritic spines. Capping of the pointed end of actin filaments is likely important for the cytoskeletal rearrangements underlying postsynaptic function.

Kristin Limpose, CB **Poster #13**

REGULATION OF THE NTHL1 BASE EXCISION REPAIR GLYCOSYLASE AND GENOME MAINTENANCE: IMPLICATIONS IN CANCER

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Dysregulation of DNA repair and genome maintenance pathways contribute to cancer by increasing levels of DNA damage. Unrepaired DNA damage results in the accrual of mutations and genomic instability. Base Excision Repair (BER) is the main pathway for repair of non-bulky DNA base damage, and is initiated by *N*- glycosylase proteins. A subset of *N*-glycosylases are bifunctional and contain an AP lyase function that cleaves the AP site phosphodiester backbone resulting in a single strand break. Therefore the intermediate steps to the BER pathway are forms of DNA damage that must be efficiently processed. Proper regulation of BER initiating steps is essential for preventing BER intermediates from becoming toxic or mutagenic. Multiple tumor types exhibit overexpression of BER glycosylases, but whether glycosylase overexpression creates an imbalance in the number of BER intermediates that result in genetic instability remains to be examined. Employing the bifunctional NTHL1 glycosylase, as a paradigm in the non-tumorigenic cell line, HBEC 3KT, we show that overexpression of NTHL1 induces genomic instability and results in an increased number of micronuclei per binucleated cell. Overexpression of NTHL1 also produced soft agar clone formation. Future experiments will focus on the mechanism(s) that contribute to the acquired genomic instability seen with NTHL1 overexpression.

Edward Quach, BCDB **Poster #14**

PREDICTING IVIG RESISTANCE IN IMMUNE THROMBOCYTOPENIC PURPURA

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Immune thrombocytopenia (ITP) is a common cause of thrombocytopenia in children and adolescents. Patients with ITP present with varying degrees of bleeding, including life-threatening intracranial hemorrhage. Currently, the standard first-line treatment for pediatric ITP is intravenous immunoglobulin (IVIG). However, approximately 20% of ITP patients are not responsive to IVIG treatment. Using IVIG to treat patients who do not respond prolongs thrombocytopenia and puts these patients at greater risk for bleeding. Unfortunately, to date there are no reliable predictors of IVIG resistance. An assay that could predict the efficacy of IVIG treatment would be highly beneficial to patient care. Based on our recent elucidation of the molecular mechanism of platelet clearance that links certain anti-platelet antibodies (Abs) to IVIG resistance, we have designed a novel flow cytometry-based assay. Beads coated with antigens for Abs serve as a stand-in for platelets, allowing us to test a patient's plasma for thrombocytopenia-inducing Abs. This assay distinguishes monoclonal Abs that rapidly clear platelets in an IVIG-resistant manner from those that do not, even when both Abs target the same epitope protein on the platelet surface. Utilizing this assay, we have shown promising results which will serve as a spring-board into pilot clinical studies.

Lauren Rusnak, CB **Poster #15**

TUMOR SUPPRESSIVE ROLE OF LATS2 IN LUNG CANCER THROUGH THE REGULATION OF ASK1 ACTIVITY AND DOWNSTREAM SIGNALING

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Large tumor suppressor kinase 2 (LATS2) is commonly lost or downregulated in lung cancer. Lower LATS2 expression correlates with a worse prognosis in patients, highlighting the clinical importance of this protein to human cancer. LATS2 suppresses growth by inhibiting oncogenic YAP in the Hippo pathway. However LATS2 is predicted to have Hippo-independent roles. Thus, identifying new binding partners of LATS2 could provide insight into new LATS2-mediated pathways and biological processes. To this end, we employed a high-throughput screen approach to detect binding partners of LATS2. The resulting data revealed the novel interaction between LATS2 and apoptosis signal-regulating kinase 1 (ASK1). ASK1 is an important mediator of the cell stress response and promotes downstream signaling to activate p38 and JNK to induce cell death. We propose that the LATS2/ASK1 PPI is a tumor suppressive interaction where LATS2 engages ASK1 to induce apoptosis. Our results showed that co-expression of LATS2 and ASK1 increases ASK1 downstream signaling. Interestingly, this effect is independent of LATS2 kinase activity, suggesting a non-enzymatic regulation of ASK1 activation. Our data suggest a model where LATS2 acts as a tumor suppressor by both inhibiting growth via YAP and promoting apoptosis through ASK1.

Brittany Phillips, GMB **Poster #16**

MUSCLE-SPECIFIC *PABPN1* REGULATION

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Muscle diseases differentially affect subsets of skeletal muscles and the mechanisms underlying muscle specificity are largely not understood. Oculopharyngeal muscular dystrophy (OPMD) affects muscles of the eyelid, pharynx, and proximal limbs causing ptosis, dysphagia, and limb weakness. Patients have a dominant GCG triplet repeat expansion mutation in the gene *PABPN1*, resulting in a modest alanine expansion in the PABPN1 protein. Although *PABPN1* is ubiquitously expressed and regulates critical aspects of pre-mRNA processing such as polyadenylation, OPMD is a muscle-specific disease. We have found that wild type PABPN1 protein and mRNA levels are low in muscle, with even lower levels in OPMD-affected muscles. Muscle-specific destabilization of *Pabpn1* mRNA contributes to low protein levels. Luciferase assays show this destabilization is mediated through the *Pabpn1* 3'UTR and that an AU-rich element (ARE) within the 3'UTR contributes to transcript stability. We have shown that HuR, a canonically stabilizing RNA binding protein that binds AREs, interacts with the *Pabpn1* 3'UTR. Interestingly, HuR is present at low levels in muscle and preliminary knockdown experiments show that HuR destabilizes *Pabpn1*. These data provide initial insight into PABPN1 regulation in muscle and suggest specific pathways that could be modulated to potentially ameliorate symptoms of OPMD.

Scott Wilkinson, CB **Poster #17**

LKB1 KINASE-DEPENDENT AND -INDEPENDENT DEFECTS DISRUPT POLARITY AND ADHESION SIGNALING TO CREATE A UNIQUELY INVASIVE AMOEBOID CANCER CELL

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LKB1 is the 3rd most-commonly mutated gene in lung adenocarcinoma, with the majority of mutations being truncations disrupting kinase activity and removing its C-terminal domain (CTD). Since LKB1 inactivation drives cancer metastasis in mice, we identified how domain-specific LKB1 inactivation impacts lung cancer 3D invasion. Cells re-expressing wildtype LKB1 or CTD alone exhibited mesenchymal polarity with strong directional persistence, which is completely abrogated upon farnesylation loss. Since the CTD is kinase-dead, these data highlight a farnesylation-dependent but kinase-independent regulation of polarity and directionality during invasion. We then examined how farnesylation regulates polarity, and now show rescuing RhoA activity in farnesylation-compromised cells restores this polarization. Importantly, activating RhoA in the absence of LKB1 fails to restore mesenchymal polarity, indicating a region of LKB1 is necessary for the regulation of polarity. Inverse of polarity, LKB1 signals to MARK1 in a farnesylation-independent but kinase-dependent manner to repress FAK, which represses collagen remodeling during 3D invasion. Since LKB1 frequently undergoes truncations affecting farnesylation and kinase activity, cancer cells with LKB1-loss would exhibit unique amoeboid morphologies with hyperactive FAK and the ability to remodel collagen. Together, these data suggest that a combination of kinase-dependent and -independent defects create a uniquely invasive cell upon LKB1 inactivation.

Kameryn Butler, GMB **Poster #18**

EVALUATION OF VARIANTS FROM EPILEPSY PATIENTS REFERRED FOR GENETIC SCREENING

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Epilepsy is a neurological disorder characterized by recurrent, unprovoked seizures caused by neuronal synchrony and hyperexcitability. The Emory Genetics Laboratory (EGL) has developed a targeted sequencing library comprising 110 known and candidate epilepsy genes referred to as the 'epilepsy and seizure disorder panel' (ESDP). The ESDP is derived from a larger mendeliome library of evidence-based disease genes, making this a valuable resource for identifying putative disease-causing alleles as well as new disease associations. Individuals referred to the EGL for genetic testing are typically affected with severe childhood epilepsy. We are utilizing this rich source of available sequence data to identify new disease-causing variants. From the examination of nearly 250 cases, we identified five novel variants in the voltage-gated sodium channel *SCN8A*, which is associated with early infantile epileptic encephalopathy and intellectual disability. Studies are underway to confirm pathogenicity and investigate the functional consequences of these variants. Missense changes at conserved positions in *SCN8A* were observed infrequently in individuals referred for genetic screening on panels other than the ESDP. In addition to the *SCN8A* variants, we identified new pathogenic or likely pathogenic variants in 29 other genes from the ESDP, accounting for approximately 30% of the cases screened on the ESDP.

Valentina Gonzalez-Pecchi, CB **Poster #19**

TARGETING MYC THROUGH ITS INTERACTION WITH THE EPIGENETIC REGULATOR, WHSC1L1/NSD3

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MYC is a mayor oncogene over-expressed in over 50% of human cancers. It is a transcriptional regulator and has been well validated as a cancer drug target. However, targeting c-MYC has been highly challenging with little progress. Because c-MYC function is controlled by signaling proteins, one alternative approach is to discover critical regulators of c-MYC and inhibit c-MYC using protein-protein interactions (PPI) disruptors. Towards this goal we utilized a high-throughput PP technology and identified a novel interaction between c-MYC and an epigenetic regulator, NSD3. NSD3 is a lysine histone methyltransferase that methylate's histones to control transcriptional program. NSD3 is commonly amplified in cancer, suggesting a potential oncogenic role. We validated the interaction between c-MYC and NSD3 by lysate based assay and endogenous co-immunoprecipitation. By mutagenesis studies, the domains that mediate the interaction of c-MYC and NSD3 have been identified, which provides a tool for functional exploration of this interaction in driving tumorigenesis. Interestingly, over-expression of c-MYC and NSD3 show changes in histone methylation pattern and in cell survival, possible reprogramming histone epigenetic signature of cells affecting cell growth pattern. My work identified a critical link between oncogene and epigenetic modulators, and could lead to new therapeutic strategies to target c-MYC driven tumors.

Crystal Grant, GMB **Poster #20**

COMPARISON OF METHODS FOR METHYLATION ANALYSIS

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As part of a larger pilot study, I will be characterizing methylation patterns of blood samples from a large human cohort with the goal of studying how methylation and its associated changes with aging mediate disease development. Whole Genome Bisulfite Sequencing (WGBS) data is considered to be a gold standard for characterizing methylation patterns, but this method is costly for large cohorts. In order to find the most cost-efficient method to perform methylation analysis, without sacrificing accuracy, I will be performing a small pilot study in which I will compare Methylated DNA ImmunoPrecipitation sequencing (MeDIP-seq) data and low coverage Bisulfite Sequencing (BS-seq) data of H1 human embryonic stem cells to the WGBS data obtained from our sequence library. Our goal is to assess the data quality and cost of performing both methods in order to decide which approach to take in our larger study.

Yun Wei, CB **Poster #21**

INDUCING MEDULLOBLASTOMA TUMOR CELL DEATH BY DISRUPTING I2PP2A'S INHIBITION OF DEATH EXECUTOR NME1

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Medulloblastoma is a pediatric brain tumor which arises in the cerebellum and is the most common childhood central nervous system malignancy. Approximately 30% of human MB show aberrant Sonic Hedgehog (SHH) signaling. Although there are drugs available that target Smoothened activity, the amplification of downstream Smo targets renders them useless in many cases. Thus, novel therapeutic modalities and targets are needed for treatment of these tumors. Utilizing the NeuroD2-SmoA1 mouse model, which recapitulates human medulloblastoma, I have been studying the nuclear oncoprotein, I2PP2A, also known as endogenous inhibitor 2 of phosphatase 2A. Analysis of a large gene expression database of medulloblastoma patients revealed an upregulation of I2PP2A mRNA in patients of all four subclasses compared to non-tumor samples, indicating the relevance of this gene in promoting medulloblastoma. I2PP2A inhibits apoptosis by binding to non-metastasis marker NME1, retaining it in the cytosol and preventing its DNAse activity. Interestingly, I have found high levels of both I2PP2A and NME1 in cytosol of mouse SmoA1 medulloblastomas, and these two proteins showed a similar cellular distribution pattern. Based on these data, I hypothesize that the death executor NME1 might be inhibited by cytosolic I2PP2A, causing the abrogation of medulloblastoma tumor cell death. My future plans are to (1) determine the role of this interaction between I2PP2A and NME1 in medulloblastoma, and (2) to identify the mechanisms driving NME1's subcellular localization. Ultimately I hope to extrapolate this research into a novel therapy designed targeting I2PP2A:NME1 interactions in SHH medulloblastoma and other subtypes of medulloblastoma.

George Inglis, GMB **Poster #22**

EVALUATING NOVEL TRANSCRIPTIONAL AND TRANSLATIONAL CONTROLS OF THE VOLTAGE-GATED SODIUM CHANNELS *SCN1A* AND *SCN8A*

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Dravet Syndrome (DS) is a severe, early-onset epileptic disorder caused by loss of function mutations in the voltage-gated sodium channel (VGSC) alpha subunit gene *SCN1A* (encoding $Na_v1.1$ channels). Increasing *SCN1A* expression and downregulation of the VGSC alpha subunit *SCN8A* (encoding $Na_v1.6$ channels) have both been demonstrated to ameliorate seizure activity in a mouse model of DS. However, while promoters and putative regulatory elements have been identified for both *SCN1A* and *SCN8A*, little progress has been made in elucidating associated regulatory factors. We have identified two proteins that may be able to specifically regulate these two channels: the VGSC β2 subunit intracellular domain (β2-ICD) and Pumilio-2 (PUM2). β2-ICD has been previously demonstrated to translocate to the nucleus and increase *SCN1A* expression and $Na_v1.1$ levels, though the underlying mechanism is unknown. PUM2 represses translation of *SCN8A* transcripts and can bind mRNA of a *SCN1A* inhibitor, though the functional significance of this interaction is unknown. We have begun to functionally evaluate these two proteins in a neuronal cell culture model to confirm their specific activity and elucidate their underlying mechanisms. This study will expand the current understanding of VGSC regulation and identify novel proteins that may serve as potential therapies for DS patients.

Alice Cho, IMP **Poster #23**

ROBUST MEMORY RECALL RESPONSE TO INFLUENZA VACCINATION IN SPITE OF B-CELL DEPLETION THERAPY

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Pemphigus is an autoantibody-mediated disorder targeting epithelial adhesion proteins, causing painful, disfiguring blisters and sores on the skin and mucosal membranes. Pemphigus can be treated with the monoclonal antibody therapy Rituximab, which efficiently depletes peripheral B-cell subsets while having no effect on long-lived plasma cells. Little is known about antigen-specific B cell responses during or after recovery of the B-cell compartment. In this study, pemphigus patients were vaccinated against influenza at various time points after B cell-ablation therapy to analyze their immune responses to the vaccine. Surprisingly, patients were able to mount potent plasmablast responses to the vaccination, comparable to those seen in healthy control vaccinees. While almost all patients lacked detectable influenza-specific memory B cells prior to vaccination, high levels of somatic hypermutation of the plasmablasts suggest that responses did originate from memory B cells. Thus, we propose that tissue-resident memory B cells can resist Rituximab-mediated depletion and may be the source for this potent recall response. Continued studies of this patient population will enable us to address fundamental questions about B-cell biology, as well as the clinically relevant issue of the impact of Rituximab on pre-existing memory B cells.

Christopher Rounds, GMB **Poster #24**

THE CONSERVED INTELLECTUAL DISBILITY RNA BINDING PROTEIN DNAB2 MAY REGULATE NEURONAL PROTEIN TRANSLATION IN CONJUCTION WITH ATAXIN-2

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Intellectual disability refers to a common class of etiologically heterogeneous neurodevelopmental disorders. Recently, we have shown that one form intellectual disability is caused by loss-of-function mutations in *ZC3H14*, a gene encoding a ubiquitously expressed RNA binding protein whose molecular function, protein binding partners, and RNA targets are largely unknown. To better understand the function of human ZC3H14, we have begun to dissect the function of its *Drosophila* ortholog dNab2. Here we present evidence that dNab2 may play a crucial role in controlling neuronal protein translation through, in part, its interactions with the neuronal translational regulator Ataxin-2 (Atx2). Loss-of-function alleles of *Ataxin-2* ameliorate effects of dNab2 loss or overexpression on survival, locomotion, and neuromorphology, implying that dNab2 and Atx2 may interact in nuanced ways to regulate these processes. Moreover, we find that tissue-specific neuronal knockdown of dNab2 elevates expression of a translational reporter corresponding to Ca^{2+}/calmodulin-dependent kinase II (CaMKII), an Atx2 target, without altering expression of a control simian virus 40 reporter. Taken with other preliminary results, these data provide insight into ZC3H14-linked intellectual disability, supporting a role for ZC3H14 ortholog dNab2 in regulating translation of specific neuronal transcripts through, at least in part, molecular interactions with the neurodegenerative-disease-linked protein Atx2.

Ching-wen Chen, IMP **Poster #25**

THE INHIBITORY ROLE OF 2B4 ON CD4+ T CELLS CONTRIBUTES TO MORTALITY IN SEPSIS

Ching-wen Chen, Danya Liu, Rohit Mittal, Craig Coopersmith, Mandy Ford

Sepsis is a life-threatening disease characterized by a dysregulated systemic inflammatory response to infection. The immunopathogenesis of sepsis occurs in two stages: an early hyper-inflammatory state followed by a hypo-inflammatory phase during which coinhibitory molecule signaling can dampen both innate and adaptive immune responses. 2B4 is a coinhibitory molecule expressed on NK cells and memory T cells, the function of which has yet to be explored in sepsis. Here, wild-type (WT) mice were subjected to polymicrobial sepsis via cecal ligation and puncture (CLP) and expression of 2B4 in sepsis was assessed. WT mice showed a marked 2B4 upregulation on T cells 24-hours post-CLP, but no change within the NK cell compartment. Importantly, 2B4-/- mice were significantly protected from death during sepsis as compared to WT controls. Furthermore, T cells from septic 2B4-/- animals exhibited higher IFN-gamma secretion compared to T cells from septic WT mice. To investigate the role of 2B4 on individual cell populations, CD4+ T cell-specific or CD8+ T cell-specific 2B4 knockout mice were generated. While CD8-specific 2B4 knockout did not affect survival, CD4-specific 2B4 knockout significantly improved survival after CLP, suggesting that expression of inhibitory 2B4 molecules on CD4+ T cells contributes to septic mortality.

Marko Bajic, GMB **Poster #26**

UNDERSTANDING THE CELL TYPE-SPECIFIC RESPONSE OF ALFALFA ROOTS TO FLOOD STRESS

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Plants are stationary organisms that must constantly respond to a changing environment in order to survive. Primary detection and response to flood stress occurs in the roots. However, how the specific cell types of the roots respond to these stresses is not completely understood. We have utilized two techniques, INTACT (Isolation of Nuclei TAgged in specific Cell Types) and TRAP (Tagged Ribosome Affinity Purification), to characterize the transcriptional and translational response of *Medicago truncatula*, alfalfa, roots to submergence stress. The nuclei and translating ribosomes are expressed in specific cell types of the root using *Arabidopsis thaliana* promoters. Our results show that all of the *Arabidopsis* promoters, with the exception of *At*WOX5, had the same localized expression in *Medicago* as in *Arabidopsis*. We also show that INTACT and TRAP can be performed using *Medicago* tissues, which has not been shown before. We are currently characterizing the response of alfalfa root cells to 2 hours of submergence stress. Our results will be compared to parallel experiments performed in tomato and rice. The long-term goal of this research is to establish a comprehensive understanding of crop stress response and to use this information to develop hardier crops.

Christopher Lewis, IMP **Poster #27**

EXOSOMES, ARYL HYDROCARBONS AND MESENCHYME: TOWARDS A CELL-FREE CELL THERAPY

Christopher N. Lewis A.B.[1], Raghavan Chinnadurai Ph.D.[2], and Jacques Galipeau M.D.[2,3]
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Our lab is directing a number of clinical trials using mesenchymal stem cells (MSCs) from our human patients as personalized cell-based therapeutics to treat immune-mediated pathologies. We and others have shown that MSC catabolism of tryptophan (Trp) by the enzyme indoleamine 2,3-dioxygenase (IDO) is a key correlate of MSC immunomodulatory potency, and hypothesized these Trp catabolites may share signaling modalities with environmentally-derived aryl hydrocarbons, known to play a carcinogenic role in human tissues via the aryl hydrocarbon receptor (AHR). AHR is a cytosolic protein expressed by a variety of cell types, that upon activation, initiates transcription at dioxin response elements. Through the use of RNA, protein and exosome-based assay systems, we show MSCs express AHR basally, suggesting IDO catabolism and AHR signal transduction are linked, as intracellular signals which deploy MSC immunomodulatory properties. The isolation of differentially-loaded and immune-modifying exosomes from cultured MSCs suggest new avenues by which cell therapies can be developed as an ex vivo but cell-free therapeutic platform. Understanding the role of AHR ligands in deploying MSC immunomodulatory exosomes will afford new insight on the ex vivo conditions that will best deploy the anti-inflammatory effects of our personalized therapies, translating to improved patient outcomes in immunologic disease.

PamelaSara Head, GMB **Poster #28**

REGULATION OF SIRT2 IN THE DNA DAMAGE RESPONSE

PamelaSara Head and David Yu

DNA Damage Response (DDR) pathways are critical for maintaining genome integrity and preventing disease including cancer and premature aging. The mechanisms mediating the activities of the DDR and how their dysregulation leads to genomic instability and a tumor permissive phenotype are not fully understood. Histone deacetylase, Sirtuin 2 (SIRT2), is implicated as a tumor suppressor. Genetic loss of *Sirt2* results in both genomic instability and specific murine breast and liver tumors. Utilizing a proteomic approach by mass spectrometry of SIRT2 purified from cells, I found that SIRT2 is differentially phosphorylated at specific serine and threonine residues in a DNA damage dependent manner. In addition, SIRT2 interacts with a number of proteins involved in the DDR, including DNA dependent protein kinase, catalytic subunit. I hypothesize that SIRT2 is regulated by phosphorylation in response to DNA damage, which is critical for its functions in the DDR. Completion of this research will delineate how SIRT2 is regulated in the DNA Damage Response and the significance of its interaction with DNA-PKcs thus providing novel insights into how SIRT2 maintains genome integrity and how loss of SIRT2 leads to a tumor permissive phenotype.

David Holthausen, IMP **Poster #29**

USING FROG PEPTIDES TO COMBAT INFLUENZA VIRUSES

David J. Holthausen[1], Sanil George[2], and Joshy Jacob[1].
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Frogs and toads secrete a tremendous amount of biologically active host defense peptides from their skin as a means to protect themselves against microbes and predators. The quantity and varying repertoire of these secreted peptides, which dwarf mammalian analogues, account for 20% of all known host defense peptides. The abundance and breadth of these peptides in tandem with the non-invasive, non-harmful collection methods, make these amphibians excellent targets for the discovery of novel peptide drug therapies. Previous studies have shown that these peptides can be effective against gram-negative and gram-positive bacteria, mycobacteria, enveloped viruses, fungi, and transformed or cancerous cells. Given the untapped potential for frog skin peptides as anti-viral therapies, we assessed a library of novel host defense peptides from the skin of *Hylarana malabarica*, a fungoid frog which resides in the mountainous Western Ghats of India against the Influenza virus. Our studies indicate peptides from *H. malabarica* that are able to act against influenza in vitro, as well as show promise as potential candidates for in vivo therapies.

Robert Haines, GMB **Poster #30**

ABERRANT EPIGENETIC PROGRAMMING OF B CELL SUBSETS IN SYSTEMIC LUPUS ERYTHEMATOSUS REVEALED BY DNA METHYLOME AND TRANSCRIPTOME PROFILING

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Systemic lupus erythematosus (SLE) is due in part to B cell defects that lead to the production of autoantibodies[1,2]. Through high-dimensional flow cytometry, expanded subsets of distinct B cell populations in SLE patients were identified. A pathogenic role for epigenetic programming in the expanded B cell populations and SLE disease etiology is unknown[3-6]. The epigenomic programming of B cell subsets in HC and SLE patients was characterized using RNA-seq and Reduced Representation Bisulfite Sequencing (RRBS) to assay the transcriptome and DNA methylation states, respectively. Distinct disease-dependent signatures were identified and included an up regulation and demethylation of interferon-regulated genes across SLE cell types. Additionally, naïve B cell subsets in SLE patients displayed an altered epigenetic signature, suggesting these cells developed in an environment that promotes pathogenic programming. These results identify loci that may contribute to the expansion of B cell subsets in SLE and further our understanding of the molecular programming of human B cell subsets.

Emily Cartwright, IMP **Poster #31**

DYNAMICS OF CD4+ T MEMORY STEM CELLS DURING SIMIAN IMMUNODEFICIECNY VIRUS INFECTION AND ANTIRETROVIRAL THERAPY IN RHESUS MACAQUES

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Disruption of memory CD4+ T cell homeostasis is a hallmark of pathogenic HIV and SIV infections. In particular, perturbation of the less differentiated CD4+ T memory stem cells and central memory T-cells (T_{SCM} and T_{CM}, respectively) distinguishes pathogenic SIV infection of rhesus macaques (RM) from non-pathogenic SIV infection. However, little is known about what effect antiretroviral therapy (ART) has on the dynamics and homeostasis of CD4+T_{SCM}. This study sought to understand the effects of ART on CD4+T_{SCM} during SIV infection of RM, including overall number, expression of the SIV-co-receptor CCR5, proliferation, and expression of PD-1. We found a partial restoration of CCR5+CD4+T_{SCM} in ART-treated SIV-infected RMs. However, the frequency of proliferating CD4+T_{SCM}, which increases during chronic infection, remains somewhat elevated during ART. Interestingly, while ART reduces the expression of PD-1 on almost all subsets of CD4+ memory T cells in PBMC and lymph nodes, CD4+T_{SCM} retain elevated levels of PD-1 in the lymph nodes. Taken together, these data show that though ART is effective at reducing viral load >99.9%, its effect on recovering CD4+T_{SCM} homeostasis is only partial.

Sara Fielder, GMB **Poster #32**

SEX SPECIFIC DIFFERENCES IN *C. elegans* MEIOTIC SILENCING

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Meiotic synapsis is a conserved process that is required for proper pairing and segregation of homologous chromosomes in both oogenesis and spermatogenesis. My lab previously showed that male *C. elegans* (karyotype XO) target the unpaired X for heterochromatin assembly, becoming highly enriched in the repressive histone H3 modification H3K9me2. This process, called "meiotic silencing" has been observed in numerous organisms, including mammals. In mutants that specifically eliminate alignment and synapsis of just one or two homologs (e.g., *zim* mutants, which coordinate pairing of one or two chromosomes), we observed that unpaired chromosomes that lack SYP proteins also show enrichment of H3K9me2, indicating that SC assembly blocks addition of H3K9me2 to chromatin. Unexpectedly, mutants that cannot initiate synapsis do not exhibit any H3K9me2 enrichment. This indicates that there is a previously undescribed checkpoint that activates H3K9me2 targeting during early meiosis that is either not reached or is bypassed in the complete absence of synapsis. This pattern is strikingly different in males lacking synapsis: all chromosomes accumulate H3K9me2 indicating either that males lack the proposed checkpoint, or regulate the checkpoint differently.

Emily Woods, MMG **Poster #33**

THE DLT PATHWAY IS REQUIRED FOR LYSOZYME RESISTANCE IN *CLOSTRIDIUM DIFFICILE* AND IS REGULATED BY σ^V

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Clostridium difficile infects close to half a million Americans each year. Despite host production of antimicrobial peptides (AMPs), such as lysozyme, *C. difficile* is able to colonize the colon and cause disease. Previous studies have shown that the Dlt pathway, which adds D-alanine to teichoic acid, is important for *C. difficile* resistance to a variety of AMPs. We therefore investigated whether this pathway is also important for resistance to lysozyme and examined the regulation of this pathway. *dlt* and *sigV* null mutants demonstrated attenuated growth in lysozyme, suggesting that both genes are important for lysozyme resistance. In strains 630Δ*erm* and R20291, *dlt* expression and D-alanylation increased in lysozyme, but this induction did not occur in a *sigV* mutant. 5' RACE and promoter reporter fusion assays have identified regions necessary for both σ^V-dependent and σ^V-independent transcription. Additionally, we found that the *dlt* and *sigV* mutants were more virulent than the parent strain in the hamster model of infection. These findings demonstrate that σ^V-dependent induction of teichoic acid D-alanylation confers lysozyme resistance to *C. difficile* and influences virulence *in vivo*.

Annie McPherson, GMB **Poster #34**

SUMO MODIFICATION OF THE BASE EXCISION REPAIR PROTEIN, NTG1, LINKS DNA DAMAGE AND DNA DAMAGE RESPONSE

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Base Excision Repair (BER), is the evolutionarily conserved major repair pathway of oxidative DNA damage. Although the BER pathway is well defined, the regulatory mechanisms are unknown. Our studies focus on *S. cerevisiae* Ntg1, a BER protein that recognizes and excises oxidized base lesions. Preliminary data show that Ntg1 can be posttranslationally modified by sumoylation, suggesting posttranslational modification as a possible mode of regulation. To investigate the purpose of sumoylation, we created a non-sumoylatable Ntg1 variant (ntg1ΔSUMO). Cells expressing ntg1ΔSUMO fail to arrest the cell cycle in response to DNA damage. Therefore, our hypothesis is that SUMO modification of key BER proteins is required to orchestrate a proper DNA damage response (DDR). Therefore, we have employed a proteomic approach to identify proteins that interact with Ntg1 in a SUMO-dependent manner. Either Ntg1 or ntg1ΔSUMO was purified and an unbiased mass spectrometry approach was used to identify interacting proteins. We have focused on proteins that show differential interaction with wildtype and ntg1ΔSUMO. Cst6 emerged as a protein of interest as it plays a role in genome stability and interacts only with wildtype ntg1. Understanding the regulation of Ntg1 provides insight into a new mode of regulation for DNA repair systems.

Gordon Dale, IMP **Poster #35**

GENE CONVERSION IN DIVERSIFICATION OF MURINE IgM PLASMA CELLS

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Humoral immunity is marked by long-lived antibody responses against pathogens. The duration of this response is dependent on long-lived plasma cells that are primarily located in the bone marrow and are of IgG isotype. However, we have identified a population of long-lived IgM plasma cells that reside in the red pulp of the spleen that display an atypical mutation profile. We find that mutations primarily occur in the framework regions of the V_H gene unlike IgG plasma cells, where mutations are predominantly located within the complementarity determining regions (CDRs). Upon further analysis of these mutations, we find strong evidence that these mutations occur in tracts and appear to be shared between multiple IgM plasma cell clones from individual animals. Further, these tracts completely match in position and identity with other highly homologous V_H genes and therefore we posit that these mutations are not random but are templated from other V_H genes and occur through the process of gene conversion.

Amielle Moreno, NS **Poster #36**

MATERNAL EXPERIENCE AND ESTROGEN TREATMENT MODULATE THE CORE AUDITORY CORTICAL EXPRESSION OF C-FOS TITLE

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Maternal response to infants requires sensitivity to infant-specific cues and the auditory cortex (AC) is the site for infant vocalization processing. Yet the underlying molecular mechanisms responsible for rapid learning of infant vocalizations in Mothers are not fully understood. Mother mice quickly learn the behavioral relevance of pup isolation calls, while non-mothers require more social experience to display the same maternal responsiveness. We hypothesize that maternal experience and the hormonal physiological state enact molecular changes in the AC, which enhance social stimuli processing. Previous studies have determined that in response to pup-calls, the expression of the immediate early gene (IEG) *c-fos* in the primary AC is significantly lower in Mothers compared to pup-naïve virgin females. By manipulating both estrogen exposure and social experience caring for pups, we found a significant effect of estrogen exposure and pup-experience on primary AC *c-fos* expression (Hormone effect: $F_{(3,260)} = 4.76$, $p < 0.029$, Pup Experience effect: $F_{(3,260)} = 27.90$, $p< 0.0001$). The expression of estrogen treated/pup-experienced females replicates maternal IEG expression in response to behaviorally relevant social auditory stimuli. We also identified potential direct and indirect pathways by which social experience and estrogen might manipulate AC neural plasticity in our new model.

Jennifer Cosby, IMP **Poster #37**

VIRAL INFECTION DELAYS DEMYELINATING DISEASE PROGRESSION

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Multiple sclerosis is an autoimmune demyelinating disease that affects approximately 2.3 million people world-wide. Infections are often proposed as the initial trigger of disease episodes or exacerbation of ongoing disease; however, this has been difficult to demonstrate. In contrast to their role as a driver of demyelination, here we investigate the potential protective roles that infections may play. Using a well-established animal model of chronic-progressive autoimmune disease, we investigated the role lymphocytic choriomeningitis virus (LCMV) infection. Mice infected with LCMV had a delay in induction of paralysis as compared to uninfected controls with reduced numbers of myelin-specific CD4 T cells in the CNS that drive autoimmunity. The myelin specific T cells were found in the periphery suggesting that the virus impeded their trafficking to the CNS. Additionally, virus-specific CD8 T cells were found in the CNS in preference to the myelin specific CD4 T cells. Thus, in contrast to the prevailing paradigm of infections driving autoimmune disease progression, we find that viral infection can lessen acute demyelinating disease.

Amy Dunn, NS **Poster #38**

DISRUPTION OF THE SYNAPTIC VESICLE GLYCOPROTEIN 2C (SV2C) IN PARKINSON'S DISEASE

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The synaptic vesicle glycoprotein 2C (SV2C) is a vesicular protein enriched in the basal ganglia and is a genetic modifier of PD risk in smokers. To explore a potential role of SV2C in PD, we evaluated striatal SV2C expression in human PD and in mouse models of PD. Striatal SV2C was disrupted in PD and in mice overexpressing mutated alpha-synuclein. WT alpha-synuclein and SV2C coimmunoprecipitate, suggesting a direct interaction between SV2C and alpha-synuclein. To address the significance of SV2C disruption *in vivo*, we neurochemically evaluated SV2C-knockout mice. We observed a slight impairment of the vesicle to store dopamine as compared to WT animals and a reduction in electrically-stimulated striatal dopamine release. Furthermore, cells overexpressing SV2C have augmented vesicular dopamine storage. These data indicate that SV2C plays an important role vesicular function in the basal ganglia and represents a promising novel focus for the study of PD.

Madelyn Houser, IMP **Poster #39**

REGULATOR OF G PROTEIN SIGNALING 10 IMPACTS MICROBIOTA AND IMMUNE FUNCTION IN THE INTESTINE

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Research increasingly suggests a key role for the intestinal microbiota-immune axis in the development of numerous chronic inflammatory conditions and even certain age-related neurodegenerative disorders such as Parkinson's disease (PD). Sustained intestinal inflammation has been implicated in the development and progression of these conditions, and the composition of the intestinal microbiota is known to both impact and be shaped by intestinal immune activity. Consequently, investigating mechanisms that regulate intestinal immune responses and how these responses are influenced by cross-talk with the microbiota has the potential to greatly improve our understanding of the pathogenesis of many complex diseases. Regulator of G protein Signaling 10 (RGS10) is a GTPase-activating protein that has been associated with suppression of proinflammatory immune signaling in microglia, macrophages, and T cells. Mixed background C57BL/6-129 mice lacking RGS10 were shown to develop neurodegeneration reminiscent of Parkinson's disease when exposed to intraperitoneal lipopolysaccharide, a system that roughly mimics an inflammatory response to microbial factors leaking from the intestine. This finding and the known involvement of the intestine in PD prompted us to investigate the function of RGS10 in the gut, seeking to describe how its impact on microbiota and intestinal immune activity regulates inflammatory responses. Here we report that RGS10 is expressed in intestinal epithelial cells and at high levels in intestinal immune cells, and that C57BL/6 mice deficient in RGS10 are more sensitive to intestinal injury and inflammation than separately housed WT animals. We utilized 16S sequencing to reveal significant differences in the fecal microbiota composition of these RGS10-null and WT mice and identified taxa that correlate well with levels of fecal cytokines. To better define the contribution of genotype to these compositional differences, we assessed the impact of RGS10 levels on the development of the microbiota in WT, RGS10-null, and heterozygote littermates from weaning to adulthood. This study describes how RGS10-dependent regulation of the intestinal microbiota-immune axis may contribute to the maintenance of healthy intestinal function or disease.

Anzar Abbas, NS **Poster #40**

EFFECT OF QUASI-PERIODIC PATTERNS ON ATTENTION AND FUNCTIONAL CONNECTIVITY

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Functional organization of brain networks plays an important role in behavior and neurological disorders. Analysis of the dynamics of two functional networks – the default mode (DMN) and task positive (TPN) networks – has shown a dependency of attention and task performance on relative network anticorrelation. Fluctuations between these two networks have been seen to occur in humans in a continuous, quasi-periodic fashion. However, the nature of these quasiperiodic patterns (QPPs) and their effect on behavior and functional connectivity is not well understood. We hypothesize that QPPs may be playing a role in the large-scale modulation of brain activity involved in processes such as attention. We aimed to examine the relative predictive value of QPPs for attention-based tasks in humans and investigate the extent to which QPPs determine functional connectivity between areas. We show that QPP phase can serve as a predictor of vigilance and that QPPs do not differ between resting state and task-based scans. Regression of QPPs from raw BOLD signal shows deficits in functional connectivity between different brain regions. Our results establish a link between QPPs and behavior and suggest that QPP occurrence may be an underlying factor for functional network strength.

Morgan Barham, IMP **Poster #41**

PHENOTYPIC CHARACTERIZATION OF MYCOBACTERIUM TUBERCULOSIS-SPECIFIC CD4 T CELLS IN INDIVIDUALS WITH HIV CO-INFECTION

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Approximately 90% of immunocompetent individuals infected with *Mycobacterium tuberculosis* (*Mtb*) never develop symptoms of clinical disease and are considered to have latent *Mtb* infection (LTBI). However, co-infection with HIV greatly increases the risk of reactivation of LTBI and progression to TB disease. Although *Mtb*-specific T cell immunity is important in maintaining immune control of *Mtb*, the immune parameters perturbed by HIV infection and result in loss of control of LTBI have not been defined. We hypothesize that *Mtb*-specific T cell function is impaired in the setting of HIV co-infection, which contributes to increased risk of TB disease in co-infected individuals. One mechanism contributing to impaired T cell function is upregulation of immunoregulatory receptors, including PD-1, BTLA, and CTLA-4. Using blood samples from HIV-infected and uninfected adults with LTBI, we tested the hypothesis that immunoregulatory receptors are upregulated on *Mtb*-specific T cells in the setting of LTBI/HIV co-infection. PBMCs were stimulated with *Mtb* CFP-10 and ESAT-6 peptide pools, and analyzed by flow cytometry to evaluate expression of inhibitory receptors by *Mtb*-specific CD4 T cells producing IFN-γ and TNF-α. We have shown that CTLA-4 is upregulated by IFN-γ producing *Mtb*-specific CD4 T cells in HIV-infected individuals.

Dexter Myrick, NS **Poster #42**

LSD1 IS THE EPIGENETIC LINK BETWEEN PATHOLOGICAL PROTEIN AGGREGATION AND NEURONAL CELL DEATH IN AD

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Alzheimer's disease (AD) is a form of dementia that is associated with widespread neuronal cell death and the accumulation of pathological protein aggregates of β-amyloid (Aβ) and hyperphosphorylated Tau. These aggregates form the hallmark AD pathologies amyloid plaques and neurofibrillary tangles (NFTs) respectively. Nevertheless, it remains unclear how these protein aggregates lead to neuronal cell death. Recent data from our lab demonstrated that the histone demethylase LSD1 is mislocalized with NFTs in AD cases. In addition, loss of LSD1 systemically in adult mice is sufficient to recapitulate many of aspects AD. These data raised the possibility that neurofibrillary tangles lead to neuronal cell death in AD by interfering with the continuous requirement for LSD1 to repress inappropriate transcription. To further investigate this model, we will determine whether LSD1 directly interacts with normal and phosphorylated forms of Tau *in vitro*. In addition to this, we also examine whether LSD1 is enzymatically active as a demethylase at Tau lysine residues known to be methylated. Tau lysine methylation has been previously detected and is correlated with increased propensity to aggregation. Furthermore, we will take an unbiased proteomics approach to identifying alterations in histone modification in our LSD1 mutant mouse model similar to AD.

Osric Forrest, IMP **Poster #43**

HUMAN RESISTIN, A NEUTROPHIL-DERIVED METABOLIC AND INFLAMMATORY MEDIATOR CORRELATES WITH DECREASED LUNG FUNCTION AND NETOSIS IN CYSTIC FIBROSIS AIRWAY DISEASE

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Resistin, a protein initially cloned from mouse adipocytes, is a strong antagonist of insulin signaling. In humans (but not mice), it is located in both the azurophil and specific granules of PMNs and a result is elevated in metabolic and PMN-driven inflammatory disorders. Cystic fibrosis (CF) combines PMN-dominated airway inflammation with profound metabolic anomalies, but the presence of resistin in CF patients has not been studied thus far. **Methods:** We measured resistin levels using an ELISA on platelet-free plasma and airway fluid from CF patients and measured the presence of DNA extracellular traps (NETs) a marker of neutrophil activation in CF airway fluid. **Results:** Resistin levels in platelet-free plasma were significantly higher in CF subjects than in HC subjects. Resistin level in expectorated CF sputum were 2 to 3 orders (100-500 fold) of magnitude higher than in plasma. Interestingly, we found a strong negative correlation between sputum resistin and lung function (Spearman Rho=-0.81, P<10-4). Similarly resistin was also shown to positively correlate with DNA-NE complexes in CF sputum. **Conclusions:** Resistin levels are abnormal in CF plasma and strikingly elevated in CF airway fluid during chronic disease. These results suggest, as has been shown in other studies, that resistin can induce NET release and contribute disease progression in the CF airways. Mechanistic studies looking at the impact of resistin on metabolism and inflammation in CF are warranted.

Elizabeth Kline, NS **Poster #44**

THE ROLE OF LRRK2 IN INFLAMMAGING AND PARKINSON'S DISEASE

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Aging is associated with dysregulated immune function, persistent low-level inflammation, and increased susceptibility for infections and diseases such as cancer, Alzheimer's, and Parkinson's disease (PD). Leucine rich repeat kinase 2 (LRRK2) is a protein highly expressed in immune cells and thought to regulate proper T cell function and activation. Although it is unknown how LRRK2 expression changes in immune cell subsets with age, the *lrrk2* locus is associated with PD, an age-related neurodegenerative disease. We report that there is greater LRRK2 expression in T cells of subjects with PD, and that there are time dependent changes in LRRK2 expression within T cells subsets upon activation. Following stimulation, LRRK2 expression levels increase in both monocytes and T cells, with no differences between PD patients and age-matched, healthy control subjects. We have also found that during T cell proliferation, subjects with PD have greater upregulation of LRRK2 compared to healthy controls. By determining the role LRRK2 plays in modulating immune function, we will reveal opportunities to develop new therapies to treat or impede progression of age-related diseases involving immune cell dysfunction.

Sarah Connolly, IMP **Poster #45**

QUANTITATIVE REAL-TIME PCR ASSAY REVEALS DIFFERENCES IN HIV RNA QUALITY AMONG CLADE C VIRUS DONORS AND RECIPIENTS

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Acute HIV infection is characterized by a genetically uniform population of viruses, established by a single transmitted founder virus (TFV) from the donor quasispecies. By investigating samples from heterosexual transmission pairs, it is possible to define both TFV and non-transmitted variants in both the donor and recipient using single genome amplification (SGA) and sequencing. Traditionally, SGA has been achieved via a time-consuming series of cDNA dilutions, followed by gel electrophoresis. **Methods:** In order to increase the efficiency of near full-length genome SGA, we developed a clade C specific quantitative real-time PCR (qPCR) assay to quantify the amount of cDNA generated based on three specific regions of the HIV genome: *gp41*, integrase, and *gag*. We infer the quality of HIV RNA by simultaneously measuring amplicons from the 3' end, center, and 5' end of cDNA using Sybr Green fluorescent chemistry (Life Technologies) and real-time detection. All three primer sets were optimized for concentration and annealing temperature so that all three targets could be tested simultaneously on the same reaction plate, reducing time and minimizing freeze/thaw cycles, which damage cDNA. Quantity is determined based on comparison to a standard curve using the linearized plasmid of a clade C TFV clone. **Results:** In this assay, we have confirmed that plasma sample viral load is correlated to both the amount of *gag* (R^2=0.74, p=0.0006) and *gp41* (R^2=0.66, p=0.0024) detected in the cDNA, however there is a dichotomy in cDNA quality as shown by the *gag*:*gp41* ratio. We tested eleven individuals, eight recipients and three donors, with varying viral loads (range: 2060 - $16.6x10^6$ copies/mL). The ratio of *gag* to *gp41* varied for these individuals, with 7/8 recipients having the highest ratio and all 3 donors having a significantly lower ratio (two tailed t test, p=0.05). **Conclusion:** Although a significant positive correlation of *gag* and *gp41* copies to plasma viral load exist in this real-time quantitative assay, the *gag*:*gp41* ratio of cDNA may be impacted by donor/recipient status due to differences in population diversity and alternative HIV transcripts. This real-time quantitative PCR technique will be used as a tool for optimizing RNA extraction and cDNA synthesis, with the goal of increasing throughput and efficiency in amplifying near full-length genomic HIV.

Elizabeth Pitts, NS **Poster #46**

REGULATION OF GOAL-DIRECTED ACTION SELECTION BY COCAINE, MDMA, AND ORBITOFRONTAL BDNF-TRKB

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Cocaine dependence is characterized by compulsive drug use and maladaptive decision-making. Adolescents are particularly vulnerable to the effects of cocaine; for example, cocaine exposure during adolescence increases the risk of developing lifelong addictions. Previous studies have shown that subchronic cocaine exposure during adolescence, but not adulthood, results in a bias towards stimulus-driven habits in mice that persists into adulthood. Changes in Brain-derived Neurotrophic Factor (BDNF) in the orbitofrontal prefrontal cortex (oPFC) could underlie, in part, this habit bias. Here we utilized viral-mediated gene transfer to decrease the expression of *Bdnf* and, in separate mice, interfere with the activity of its high-affinity receptor tyrosine kinase receptor B (trkB) selectively in the oPFC. Both manipulations induced habit-like behavior. Next, we hypothesized that stimulating BDNF expression in the oPFC could block habits by enhancing response-outcome learning and memory. We report that 3,4-methylenedioxymethamphetamine (MDMA) increases BDNF levels in the oPFC, but not the amygdala or dorsal striatum, and also "breaks" habits resulting from adolescent cocaine exposure, as well as oPFC-selective *Bdnf* knockdown. Finally, 7,8-dihydroxyflavone (7,8-DHF), a trkB agonist, also reverses cocaine induced habits, further suggesting that BDNF-trkB systems are a point of intervention in combatting maladaptive decision making following repeated cocaine exposure.

Shardule Shah, IMP **Poster #47**

CHARACTERIZATION OF HEAT SHOCK FACTOR 1 IN RESPONSE TO PROTEASOME INHIBITION UNVEILS A NOVEL THERAPEUTIC STRATEGY FOR MULTIPLE MYELOMA TREATMENT

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Proteasome inhibitors (PIs) are highly active in multiple myeloma (MM) by affecting signaling cascades and leading to a toxic buildup of misfolded proteins. PI-treated cells also activate the cytoprotective heat shock response (HSR), including upregulation of heat shock proteins (HSPs). Here we show that HSR disruption can be achieved by inhibition of the master regulator, Heat Shock Factor 1 (HSF1). We also demonstrate differential HSF1 phosphorylation sites between treated and untreated cells. HSF1 inhibition leads to downregulation of the PI-induced HSR and additively increases cell death. In contrast, individual inhibition of most HSPs, except HSP40β, does not result in an additive death effect. However, HSP40β cannot fully account for HSF1 sensitization. To determine the mechanism of PI-induced activation, we assessed phosphorylation and found an increase in cell lines and patient samples. We determined that this change occurs primarily at S326. Prior use of HSP inhibitors in combination with PIs has been disappointing in MM therapy. A potentially more effective therapeutic strategy is shown here. Our results provide a rationale for targeting HSF1 in combination with proteasome inhibition to increase MM patient long-term survival.

Erica Landis, NS **Poster #48**

INDOOR LIGHT LEVELS INCREASES SUSCEPTIBILITY TO MYOPIA IN WILD-TYPE MOUSE MODEL

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As the prevalence of myopia nears epidemic levels around the world, researchers have turned to light level exposure as a possible prevention, noting that children who spend more time outdoors in bright sunlight are less myopic. Testing myopia susceptibility in a mouse model exposed to bright, intermediate, or low light, we show that while bright and low light, simulating sun and starlight, protect against myopia, intermediate light, simulating indoor light, increases susceptibility to myopia. Male C57BL/6J mice were exposed to light treatment beginning at post-natal day 23 (P23) and a subset were given monocular lens defocus meant to induce myopia at P28. Signs of myopia such as refractive error, which is a measure of the power of the eye (diopters (D)), and other ocular parameters were measured at P23, P28, and the final time-point P36. At P36 mice housed in bright (-2.604±0.544) and low (-1.807±0.608D) light were significantly less myopic then mice in intermediate light (-4.741±0.608; $p<0.005$). This is due to changes in dopaminergic activity in the retina, a known factor in myopia development, which is regulated by light intensity. High performance liquid chromatography (HPLC) analysis of dopamine and its primary metabolite DOPAC in experimental retinas support this conclusion.

Zachary Ende, IMP **Poster #49**

TRANSMITTED HIV-1 VARIANTS FROM ZAMBIAN COUPLES DEMONSTRATE IN VITRO FITNESS HETEROGENEITY

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In ~85% of heterosexual HIV-1 transmissions, an infected individual with a diverse viral quasispecies transmits a single viral variant, the transmitted/founder (TF). Why this genetic bottleneck occurs is unknown. These breakthrough TF viruses, unique with each transmission, may have common properties that confer a higher transmission capacity; defining viral correlates of transmission could help fill-in knowledge gaps about transmission and impact interdiction strategies. To investigate potential viral correlates of transmission, we analyzed genome-length viral sequences from 6 heterosexual transmission events from an HIV discordant couple cohort in Zambia. We constructed transmitted/founder (TF) clones and a panel of non-transmitted (NT) clones from each partner's quasispecies near the date of infection. We measured genotypic (HIV sequence data) and phenotypic (*in vitro* assays) traits potentially relevant to transmission.Consensus-like genomes (p=0.047) relatively sensitive to donor antibodies (p = 0.031) were selected for during transmission, supporting previous observations in this cohort. However, TF variants did not demonstrate increased *in vitro* viral fitness by particle infectivity, viral replicative capacity, or resistance to interferon-alpha compared to the matched NT variants suggesting HIV-1 heterosexual transmission is permissive to substantial phenotypic diversity.

Jacob Billings, NS **Poster #50**

MULTISPECTRAL AND MULTISPATIAL WHOLE-BRAIN FUNCTIONAL CONNECTIVITY NETWORKS

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Functional connectivity (FC) analysis of functional magnetic resonance imaging (fMRI) data provides a window into the brain's spontaneous organization. But whereas the brain—an interwoven collection of communicating neurons—is organized in a multiscalar manner, it has been difficult to represent the brain's multiscalar organization using traditional FC analysis techniques. The present manuscript demonstrates the application of the wavelet packet transform followed by hierarchical clustering to delineate networks across spatial and spectral scales. Networks from 112, 30, 5, and individual volunteers were examined at complimentary spatial scales to depict network variation across spectral systems. Noisy information at high frequencies in the fMRI data were readily identified using an entropy metric. A mutual information-based criteria showed that FC networks organize into clearly defined spectral systems: one comprising all wavelet packets that include DC frequencies, another in a traditionally studied low-frequency oscillation range (LFO, 0.01 to 0.1 Hz), a third covering a transitional band (0.1 to 0.2 Hz), and a fourth covering all higher frequencies (0.2 to 0.776 Hz). Network variations within the LFO range resemble known functional networks particularly well. These trends show clearly among group datasets, and thus lend support to observations made about individual datasets rendered at 'optimal' scales.

Elizabeth Littauer, MMG **Poster #51**

PREGNANCY MODULATES CELLULAR AND HUMORAL IMMUNE RESPONSES TO H1N1 INFLUENZA INFECTION

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The 2009 H1N1 flu pandemic demonstrated that healthy pregnant women who became infected with influenza were at high risk for acute respiratory distress syndrome and pregnancy complications, leading to increased incidence of hospitalization, preterm births and low birth weight neonates. We generated a pregnant mouse model which recapitulates clinical phenotypes described during influenza infection of pregnant women. Pregnant mice sublethally infected ($0.5xLD_{50}$) with pandemic H1N1 A/California/07/09 show higher viral titers and delayed viral clearance relative to non-pregnant mice, and increased incidence of stillbirths and small-for-gestational-age offspring. Lymphocytes isolated from lungs and spleens of infected pregnant and non-pregnant mice at days 7 and 14 post infection were analyzed for H1N1 A/Ca/07/09 specific IL-4 and IFN-γ responses in ELISPOT assays. Pregnancy resulted in delayed flu-specific cytokine secretion at the site of infection, indicating systemic dysregulation of anti-viral responses. We hypothesize that hormonal regulation during pregnancy dampens cellular immune functions during influenza infection, resulting in increased viral pathogenesis and poor outcomes for offspring. In immunity studies, pregnant mice generated equivalent HAI sera and increased IgA antibody secreting cells (ASC) in the lungs 6 weeks post infection, indicating a potential role in pregnancy favoring the development of mucosal immunity after prolonged antigen exposure.

Julianne Freeman, NS **Poster #52**

NEURONAL SUBSTRATE SUPPORTING HAND FUNCTION IN CHRONIC STROKE PATIENTS WITH INCOMPLETE MOTOR RECOVERY

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Stroke often impacts function of the primary motor cortex (M1) and its corticospinal projections (CST) resulting in incomplete recovery of hand function. The neuronal substrate supporting hand function in stroke is not well understood. We studied the relationship between compromised hand function and ipsilesional M1 (il-M1) and CST (il-CST) organization in sixteen patients with chronic stroke involving M1 or/and CST. Hand function was quantified by peak acceleration of wrist extension movements. M1 and CST organization was determined using magnetic resonance imaging (MRI) and transcranial magnetic stimulation (TMS). An input-output curve of the il-M1 hand area was acquired with TMS. A Boltzmann function was fitted to extract maximum motor evoked potential amplitude (MEPmax) and slope-parameter. M1 thickness (FreeSurfer) and CST fractional anisotropy (FA-value) (TRACULA) were also determined. There was a significant correlation between hand function and the slope-parameter for patients with subcortical ($p=0.0252$) but not cortical stroke ($p=0.9553$). FA-value and M1 thickness of the il-M1and il-CST were smaller when compared to contralesional M1 and CST, but no significant correlations were found with hand function. The lack of strong correlations between hand function and measures of il-M1and il-CST organization indicates that hand function in chronic stroke likely depends on additional brain areas.

Erica Bizzell, MMG **Poster #53**

CHARACTERIZATION OF THE SECRETED SERINE PROTEASE Rv2223c OF *MYCOBACTERIUM TUBERCULOSIS.*

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Mycobacterium tuberculosis (*Mtb*) has evolved multiple strategies to evade host immune defenses and replicate within immune cells. These include alteration of its complex cell wall during intracellular growth, and secretion of effectors that modulate immune responses and enhance pathogen survival. Several pathogenic bacteria use extracellularly secreted proteases to regulate processes ranging from repression of cytokine production to degradation of surface-associated host proteins. While Mtb encodes several putative secreted proteases, their functions are poorly understood. One such protease, Rv2223c, is transcribed from a predicted operon with a cell-wall associated serine protease, Hip1 (Rv2224c) with which it shares 52% amino acid identity. Hip1 is involved in modification of the *Mtb* cell wall during infection, and functions by dampening normal immune responses to *Mtb* infection. We have observed that Rv2223c is secreted from mycobacterial cells and undergoes further autoproteolytic cleavage upon secretion. Additionally, we have found that Rv2223c interacts with the Hip1 physiological substrate, GroEL2, suggesting potentially overlapping or cooperative functions of these proteases. Biochemical analyses of Rv2223c to determine enzymatic activity, as well as studies exploring the effects of Rv2223c on *Mtb* infection of host cells are currently being conducted. These studies will provide insights into the molecular functions of Rv2223c in *Mtb* pathogenesis.

Laura Butkovich, NS **Poster #54**

A NOVEL TRANSGENIC MOUSE MODEL TO INVESTIGATE PARKSINSON'S DISEASE-LIKE α-SYNUCLEIN PATHOLOGY IN NORADRENERGIC NEURONS

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To date, we have lacked suitable models to understand how α-synuclein (αsyn) pathology specifically affects noradrenergic systems in Parkinson's disease (PD), and whether noradrenergic neurons are vulnerable to αsyn pathology. While cell loss and αsyn aggregates in the substantia nigra pars compacta (SNpc) are a major hallmark of PD, pathology in the locus coeruleus (LC) is commonly as severe, and may even precede that found in the SNpc. Increased expression of αsyn is a factor in its aggregation, as heritable triplication mutations in the *SNCA* gene are associated with early-onset PD. While SNpc and LC pathology are frequently comparable in PD, transgenic models have previously been unable to selectively target αsyn overexpression to the LC, limiting our understanding of how αsyn pathology affects noradrenergic neurons. To examine this question, we have developed a BAC-transgenic mouse model overexpressing wild-type human αsyn under the control of the noradrenergic-specific dopamine β-hydroxylase promoter. These animals overexpress human αsyn in LC neurons. Preliminary analysis revealed human αsyn immunoreactivity and mRNA expression in noradrenergic neurons of the LC in transgenic mice, but not non-transgenic littermates. Immunofluorescent analysis demonstrated human αsyn co-labeled with synapsin, a marker of nerve terminals, in the amygdala, cortex, and hippocampus of 14, but not 3 month old transgenic mice, suggesting age-dependent transport of human αsyn to LC terminals. These results confirm that this novel transgenic mouse model overexpresses human αsyn in noradrenergic cell bodies and projections, and may provide insight into the mechanisms of PD-like αsyn aggregation and spread, as well as a platform to test future therapeutic strategies.

Michele Daly, MMG **Poster #55**

DUAL ANTI-HIV MECHANISM OF CLOFARABINE

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Clofarabine is an FDA approved ribonucleotide reductase inhibitor, which has shown potent antiretroviral activity in transformed cell lines. Here, we explore the potency, toxicity and mechanism of action of clofarabine against HIV-1 in the human primary HIV-1 target cells: activated $CD4^+$ T cells and macrophages.Our data shows that clofarabine is a potent HIV-1 inhibitor in activated $CD4^+$ T cells and macrophages. Due to its minimal toxicity in macrophages, clofarabine displays a selectivity index over 300 in this nondividing cell type. The anti-HIV-1 activity of clofarabine correlated with a significant decrease in both cellular dNTP levels and proviral DNA synthesis. Additionally, we observed that clofarabine triphosphate was directly incorporated into DNA by HIV-1 reverse transcriptase and blocked processive DNA synthesis, particularly at the low dNTP levels found in macrophages. Taken together, these data provide strong mechanistic evidence that clofarabine is a dual action inhibitor of HIV-1 replication that both limits dNTP substrates for viral DNA synthesis and directly inhibits the DNA polymerase activity of HIV-1 reverse transcriptase, particularly in non-dividing HIV-1 target cells.

Maylen Perez Diaz, NS **Poster #56**

THE HIGHLY SELECTIVE 5-HT_{2C} RECEPTOR AGONIST WAY163909 REDUCES COMPULSIVE BEHAVIOR AND FOOD INTAKE IN FEMALE RHESUS MONKEYS

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Compulsivity has been linked to several types of addiction, a highly prevalent public health issue. It can be defined as a general inability to alter behavior with changing reinforcement contingencies. A switch from random or recreational use of reinforcers, such as drugs or highly palatable foods, to compulsive use is one of the hallmarks of addiction. Thus, compulsivity appears to be a core behavioral feature of addiction, although no one has evaluated this hypothesis directly. A highly selective 5-HT_{2C} receptor agonist, WAY163909 (WAY), has been shown to decrease food consumption and effectively reduce self-administration of psychostimulants. If compulsivity is a core feature of addiction, then activation of 5-HT_{2C} receptors should also reduce compulsive behavior. In order to test this hypothesis, we evaluated the effects of WAY (vehicle, 0.1mg/kg, 0.3mg/kg and 1.0mg/kg) on perseverative responding during a Discrimination Reversal Learning (DRL) task in rhesus monkeys (N=5). WAY increased correct responses ($p<0.05$), while decreasing perseverative responses ($p<0.05$). A proof-of-concept experiment was conducted to demonstrate that WAY reduces food consumption in our subjects. These results demonstrate the modulatory role that 5-HT_{2C} receptors play in both food consumption and compulsivity, which may inform the search for novel pharmacotherapies for treatment of addiction.

Kyle Gerber, MSP **Poster #57**

REGULATOR OF G PROTEIN SIGNALING 14 (RGS14) INTERACTS WITH 14-3-3 VIA AN H-RAS AND GAI-DEPENDENT MECHANISM

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RGS14 is a multifunctional protein scaffold that is known to interact with active Gαi/o via its RGS domain, Raf and active H-Ras-GTP via its R1 Ras binding domain (RBD), and inactive Gαi 1/3 via its G protein regulatory (GPR; also known as GoLoco) domain. RGS14 has been shown to suppress long term potentiation (LTP) in the CA2 region of the hippocampus, thereby regulating hippocampal-based learning and memory. The 14-3-3 family of proteins has recently been shown to be necessary for hippocampal LTP and associative learning and memory. Here we show evidence for an H-Ras and Gαi-dependent interaction between RGS14 and 14-3-3. Following overexpression of FLAG-RGS14 and 14-3-3g in HEK293T cells and pull-down of FLAG-RGS14, we observed an interaction between RGS14 and 14-3-3g only in the presence of Gαi and/or the constitutively active form of H-Ras(G12V). This interaction appears to occur within the R1 domain of RGS14, and is corroborated by mass spectrometry analysis of proteins recovered from intact cells. RGS14 interaction with 14-3-3g does not appear to be due to direct binding of Gαi or H-Ras(G12V) to RGS14. Ongoing studies will further elucidate the nature and physiological function of this interaction between RGS14 and 14-3-3g, which may give insight into the functions of both RGS14 as well as 14-3-3 in their roles in modulating synaptic plasticity in the hippocampus. *Studies supported by R01-NS037112-15 (JRH) and T32-GM008602 (KJG).*

Olivia Moody, NS **Poster #58**

EFFECT OF MUTAGENESIS OF LOOP B ON LORAZEPAM POTENTITATION OF $GABA_A$ RECEPTORS

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The molecular effects of benzodiazepines (BZDs) on γ-aminobutyric acid receptors ($GABA_ARs$) have been studied for decades since their introduction into clinical medicine in the 1960's. $GABA_ARs$ are pentameric anion channels with a high-affinity binding site for BZDs at the interface of the α+/γ2- subunits. Previous studies have isolated 6 loops (A-F) thought to be critical for forming this BZD binding pocket. To alter the structure of the BZD binding pocket, we mutated a threonine residue (T162) in Loop B of the α2 subunit to a proline (the homologous residue present in less BZD-sensitive α subunits 4-6). We predicted this α2(T190P) mutation would lower sensitivity of the receptor to BZDs. Whole-cell patch clamp recording of HEK293T cells expressing either α2β2γ2 or α2(T190P)β2γ2 $GABA_ARs$ showed no significant changes in Hill slope, maximum peak current or EC_{50} values for GABA, suggesting normal GABA function of the mutant receptors. Surprisingly, potentiation of lorazepam (1μM), a positive BZD, was increased in α2(T190P)-containing receptors relative to non-mutated receptors, though not reaching significance (p>.05). As a relatively small residue, proline may have altered the secondary structure of the BZD pocket, favoring the more efficacious binding of lorazepam but further study is needed to explore this.

Lauren Shapiro, MSP **Poster #59**

THE ANTIDEPRESSANT-LIKE UTILITY OF RHO-KINASE INHIBITION IN ADOLESCENTS IS TRKB-DEPENDENT

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Adolescence represents a critical period of neurodevelopment, defined by structural and synaptic maturation and reorganization within the prefrontal cortex. Although these processes are critical for the transition to adulthood, structural instability may open a window of vulnerability to neuropsychiatric disorders including depression. Therapeutic interventions that impact the neural remodeling that occurs during adolescence may be advantageous. We evaluated the therapeutic-like potential of the brain-penetrant Rho-Kinase (ROCKII) inhibitor, fasudil. Fasudil had antidepressant-like properties in the forced swim test in adolescent, though not adult, mice and was indistinguishable from fluoxetine and ketamine in this test. Additionally, acute fasudil decreased the latency to approach a palatable food in the novelty suppressed feeding task, a rapid antidepressant-like effect. Within the adolescent ventromedial PFC (vmPFC), fasudil increased the ratio of full-length (active) to truncated (inactive) tyrosine kinase receptor B (TrkB), and viral-mediated over-expression of truncated TrkB blocked fasudil's antidepressant-like effect. Finally, acute fasudil increased expression of the post-synaptic marker PSD-95, while decreasing dendritic spine density in adolescence, resulting in adult-like spine densities in the vmPFC. Together, our findings suggest that fasudil has rapid antidepressant-like actions in adolescents via regulation of vmPFC cytoskeletal and neurotrophin systems.

Rachel Cliburn, NS **Poster #60**

RELATIONSHIP BETWEEN ALTERED VESICULAR MONOAMINE FUNCTION AND COMPLEX BEHAVIOR

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Vesicular monoamine transporter 2 (VMAT2) is a presynaptic transmembrane protein which sequesters monoamines into the vesicular lumen to prepare for release. VMAT2-LO, -WT, and –HI mice contain 5%, 100%, and 200% of WT levels of VMAT2, respectively. We hypothesized that this variability in VMAT2 function mediates a range complex behaviors in mice, including fear behavior and the appetitive response to psychostimulants. VMAT2-LO mice display an anxiety- and depressive-like phenotype, whereas VMAT2-HI mice show improved outcomes in these tests as evidenced by reduced immobility time in a forced swim test (21.7%) and fewer marbles buried in a marble burying assay (38.7%) compared to WT mice. Furthermore, VMAT2-LO mice exhibit a 34% increase in freezing response compared to VMAT2-WT or –HI mice in a test of contextual fear conditioning. Additionally, VMAT2-HI mice display a reduced (50%) preference for a cocaine-paired context, but show no difference in cocaine-induced locomotion when compared to WT mice. Alternatively, VMAT2-display unaltered preference for cocaine at 10 mg/kg, but do display a marked increase in cocaine-induced locomotion. The ability of altered VMAT2 to influence response to psychostimulants, anxiety, depression, and fear response suggests that therapeutic approaches aimed at modifying VMAT2 function may be of benefit in these conditions.

Katherine Squires, MSP **Poster #61**

RGS14 BINDS AND COLOCALIZES WITH RAP2 TO REGULATE PC12 CELL NEURITE OUTGROWTH

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G protein coupled receptors direct many aspects of synaptic signaling and plasticity in the central nervous system, and their "off rate" is mediated by the regulators of G protein signaling (RGS). RGS14 is a multifunctional protein whose expression is limited to the hippocampal area CA2. Under typical conditions, Shaffer collateral input to CA2 synapses are resistant to LTP. RGS14 knockout rescues this suppression of LTP and enhances hippocampal-based learning and memory. The mechanisms by which RGS14 mediates suppression of LTP and learning is unknown, but several small G proteins related to LTP, H-Ras and Rap2A, are reported RGS14 binding partners. Our previous work has shown that active H-Ras-GTP complexes and colocalizes with RGS14, and that the RGS14:H-Ras-GTP complex stimulates neurite outgrowth in PC12 cells. Here we examine the functional effects of Rap2 interactions with RGS14. We find that active Rap2-GTP forms a stable complex with RGS14, and Rap2A:RGS14 complexes are regulated by Gαi in live cells. Further, RGS14 is recruited to the membrane by activated Rap2, and Rap2A:RGS14 complexes regulate neurite outgrowth in PC12 cells. While the definitive mechanism of RGS14-mediated suppression of synaptic plasticity and learning remains incomplete, here we describe evidence for the possible involvement of a specific RGS14:Rap2A signaling complex.

Termpanit Chalermpalanupap, NS **Poster #62**

EFFECTS OF HYPERPHOSPHORYLATED TAU ON LOCUS COERULEUS NEURONAL SURVIVAL AND NEURITE LENGTH *IN VITRO*

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The locus coeruleus (LC) is a brainstem noradrenergic nucleus that supplies norepinephrine to the forebrain and degenerates very early in nearly all Alzheimer's disease (AD) patients. The ubiquitous loss of the LC correlates well with increasing severity of other neuropathological hallmarks of AD such as beta-amyloid and tau aggregates and cognitive deficits. Recent studies suggest that aberrant hyperphosphorylated tau can be found in the LC of young, cognitively normal individuals in the absence of any other AD-like pathologies and prior to widespread depositions and neurodegeneration. It is unclear what effect this early presence of tau has on the function and survival of the LC, especially in conjunction with other genetic and environmental risk factors. By crossing mice expressing green fluorescent protein driven by the tyrosine hydroxylase promoter with mice expressing a form of mutant tau (P301S) that closely recapitulates AD-like pathology, we have successfully isolated and cultured primary LC neurons expressing aberrant tau to study their susceptibility to toxic challenges associated with AD *in vitro*. Preliminary analyses reveal that hyperphosphorylated tau may have deleterious effects on LC neurite length and acts in a synergistic manner with other toxins to induce LC neuronal death.

Ju Young Kim, MSP **Poster #63**

OVOL2 IS A CRITICAL REGULATOR OF ER71/ETV2 IN GENERATING FLK1$^+$, HEMATOPOIETIC, AND ENDOTHELIAL CELLS FROM EMBRYONIC STEM CELLS

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We here report that OVOL2, a C2H2 zinc finger protein, is a novel binding protein of ER71, which is a critical transcription factor for blood and vessel development. OVOL2 directly interacted with ER71, but not with ETS1 or ETS2, in the nucleus. ER71-mediated activation of Flk1 promoter was further enhanced by OVOL2, although OVOL2 alone failed to activate it. Consistently, co-expression of ER71 and OVOL2 in differentiating embryonic stem cells (ESCs) led to a significant augmentation of FLK1$^+$ cells, endothelial and hematopoietic cells. Such cooperative effects were impaired by the shRNA-mediated inhibition of Ovol2. Collectively, we conclude that ER71 directly interacts with OVOL2 and that such interaction is critical for FLK1$^+$ cell generation and its differentiation into downstream cell lineages.

Ashley Swain, NS **Poster #64**

STRUCTURAL PLASTICITY OF THE GABAERGIC PALLIDOTHALAMIC SYSTEM IN MPTP-TREATED PARKINSONIAN MONKEYS

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Parkinson's disease (PD) is characterized by loss of midbrain dopaminergic neurons, which results in an increased inhibitory outflow from the internal globus pallidus (GPi) onto the thalamus. In addition to previous reports of parkinsonism-associated changes in thalamic metabolism, neurochemistry and GABA receptor binding, our preliminary light microscopic data suggest ~20% decrease in vesicular GABA transporter (vGAT) immunoreactivity in the basal ganglia-receiving motor territory (BGMT) of the thalamus in MPTP-treated monkeys. We hypothesized that this change in vGAT immunostaining results from a decrease in the prevalence and changes in the morphology of GPi terminals in the BGMT of parkinsonian monkeys. Thus, immuno-electron microscopy was used to quantitatively assess the density and various morphometric measurements of GABAergic terminals in the BGMT. Our data extend previous findings that GPi terminals have a large size and form multiple symmetric synapses with large dendrites of BGMT projection neurons and interneurons. Preliminary evidence suggest a decreased number of synapses formed by GPi terminals in the BGMT of parkinsonian animals. Three dimensional reconstruction of individual GPi boutons will be performed to extend these preliminary observations. Studies are in progress to assess changes in the synaptic targets of GPi terminals in the BGMT of normal and parkinsonian monkeys.

Brandon Stauffer, MSP **Poster #65**

MECHANISTIC INSIGHTS INTO THE INHIBITION OF CFTR BY BACTERIAL SPHINGOMYELINASE

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Background: Chronic pulmonary diseases such as Cystic Fibrosis and COPD commonly present with persistent pulmonary bacterial infections that correspond with worsening of clinical prognosis. Sphingomyelinase (SMase), an enzyme that cleaves the membrane lipid sphingomyelin into phosphoryl choline and ceramide, is a secreted bacterial virulence factor that has been shown to inhibit CFTR chloride channel function and may contribute to the observed exacerbation of disease by dehydrating airways and facilitating bronchial plugging. The goal of this study is to determine how SMase inhibits CFTR channel activity with the hope of elucidating a novel CFTR regulatory mechanism with potential clinical importance.
Results: Our data indicate that the enzymatic activity of SMase inhibits CFTR channels at the cell membrane and does not lead to internalization. SMase does not inhibit CFTR by affecting the well-described regulatory domain. Interestingly, introducing mutations into CFTR that increase channel activity decrease sensitivity to the inhibitory effect suggesting that SMase locks channels closed. Consistent with this conclusion, Cystic Fibrosis-associated mutations that decrease channel opening increase the sensitivity of channels to inhibition by SMase.
Conclusion: Taken together, our data suggest SMase is an activity-dependent CFTR inhibitor that blocks channel activity without affecting the classic regulatory mechanism.

Joseph McMillan, PBEE **Poster #66**

EXPERIMENTAL PERTURBATIONS OF *CULEX SPP.* MOSQUITO PRODUCTIVITY AND ITS POTENTIAL IMPACT ON WEST NILE VIRUS TRANSMISSION

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Larvicides are a common tool for controlling *Culex* mosquitoes in roadside catch basins. However, whether larvicides actually reduce adult mosquito populations and mosquito-borne pathogen transmission is seldom studied. We conducted an experiment to determine the impact of larvicide applications in roadside catch basins on: *Culex spp.* larval abundance, pupal productivity, and measures of West Nile virus (WNv) amplification. During July and September 2015, 36 catch basins within Grant Park (Atlanta, GA) were treated weekly with a *Bacilluis thuringiensis israelensis* (Bti) larvicide. 7 basins within the park were sampled throughout 2015 to monitor the effect of the larvicide on *Cx. spp.* breeding populations. Gravid and CDC light traps were set throughout the park to monitor WNv infection in the adult mosquito populations. Blood samples from the park's avian reservoir population were collected to measure WNv activity. All collected adult mosquitoes were tested for WNv using virus isolation. These methods were paired with unmanipulated collections from an untreated park. Before-After-Control Intervention (BACI) analyses showed that the application of larvicides effectively reduced *Cx. spp.* breeding populations in Grant Park between the pre- and post-intervention periods. BACI analyses of *Culex spp.* population abundance between Grant Park and the untreated park were not significant.

Ayush Kishore, MSP **Poster #67**

STALK-DEPENDENT AND STALK-INDEPENDENT ACTIVITY OF THE ADHESION GPCR GPR56/ADGRG1

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The Adhesion G protein-coupled receptors (aGPCRs) comprise a poorly understood family of 33 receptors in humans. A defining characteristic of virtually all aGPCRs is the possession of the GPCR Autoproteolysis Inducing (GAIN) domain, which is found on the proximal end of the NT and controls the autocatalytic proteolysis of the receptor into an NT protomer and 7TM protomer; which can stay non-covalently associated for some time. Recently, great progress has been made to shed light upon a more detailed view of how exactly NTs inhibit aGPCR-mediated signaling. Notably, two separate groups have espoused the cryptic agonist model of aGPCR activation wherein GAIN domain cleavage unveils a new NT stalk which serves as the receptor's agonist. In this model, the stalk is a requisite agonist for aGPCR activity as deletion of the stalk resulted in a presumably dead receptor in the studies put forth by the two groups. Here we show that the stalk of aGPCR ADGRG1(G1) is indeed necessary for activation of certain pathways (activation of serum response factor, SRF) but is dispensable for others (shedding of membrane-bound TGFalpha and activation of nuclear factor of activated T cells, NFAT). These lines of evidence suggest that there may be at least two modes of activity meditated by G1: stalk-dependent and stalk-independent. Based upon our results, we propose an even more nuanced model of aGPCR activation in which the inhibitory NT modulates activity of the 7TM in two distinct ways: i) by antagonizing stalk-dependent activity by masking the cryptic stalk peptide, and ii) by directly antagonizing the inherent stalk-independent activity of the 7TM protomer.

Dawn Barnes, BCDB **Poster #68**

A NOVEL ALTERNATIVE EXON OF THE *CAENORHABDITIS ELEGANS LEV-11* TROPOMYOSIN GENE IS USED TO EXPRESS A HEAD-MUSCLE-SPECIFIC ISOFORM

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Tropomyosin (TM) is a coiled-coil dimer that binds along actin in muscle and non-muscle cells. TM is critically-involved in muscle contractility. Four *Caenorhabditis elegans* TM isoforms have been reported from a single gene, *lev-11*. We identified a novel exon (7a) that is alternative to 7b (previously 7) and cloned an isoform containing Exon 7a, termed LEV-11O. 7b was favorably-included in pharynx and main body body-wall muscles. 7a was alternatively spliced in the head. Levamisole induces hyper-contracted muscle paralysis in wild-type nematodes. An amino acid charge reversal from within *lev-11* exon 7b (LEV-11A) prevented levamisole-induced hyper-contraction in the main body but not the head; whereas an exon 7a (LEV-11O) charge reversal prevented levamisole-induced hyper-contraction in the head. These data indicate that exons 7a and 7b have non-redundant functions for muscle regulation in the head or main body. LEV-11A and LEV-11O bound actin with similar affinity and mediated inhibition of actomyosin ATPase by a TNI inhibitory peptide. LEV-11O(E196K) strongly-inhibited actomyosin ATPase even in the absence of the TNI inhibitory peptide, suggesting that enhanced inhibitory effects on actomyosin ATPase is the basis of levamisole resistance. These results suggest that *C. elegans* utilize muscle-type-specific alternative splicing to produce functionally distinct TM isoforms.

James Cordova, MSP **Poster #69**

SPECTROSCOPIC MRI IDENTIFIES REGIONS OF TUMOR INFILTRATION AND PREDICTS THE LOCATION OF TUMOR RECURRENCE IN PATIENTS WITH GLIOBLASTOMA

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T1-weighted, contrast-enhanced MRI (T1WCE) guidance of GBM surgery results in high rates of local recurrence due to tumor infiltration beyond enhancing margins. Spectroscopic MRI (sMRI) can identify tumor infiltration outside of T1WCE regions. Coupling preoperative sMRI with fluorescence-guided surgery (FGS) and quantitative histopathological analysis, tumor infiltration can be quantitatively defined in terms of metabolic abnormalities. sMRI volumes were used for surgical planning in 20 GBM patients receiving FGS. Biopsies were collected from regions with metabolic abnormalities before bulk resection. Tissue fluorescence was quantified *ex vivo* with a hand-held spectrometer, and metabolic data was sampled from sMRI volumes at the point of tissue extraction. Samples were stained for SOX2, a tumor-specific marker, and analyzed to quantify the density of SOX2 cells using automated histology image analysis. Cho/NAA, NAA, and Cho showed strong, statistically significant correlations with SOX2 density ($\rho = 0.82$, -0.50, & 0.63, respectively; $p < 0.05$). Cho/NAA and Cho also showed significant correlations with *ex vivo* fluorescence ($\rho = 0.365$ & 0.404; $p<0.05$). Lastly, pretherapy Cho/NAA abnormalities predicted the location of contrast-enhancement at tumor recurrence. The correlation of sMRI abnormalities with quantitative measures of infiltration supports the use of sMRI for identifying regions of tumor infiltration outside of T1WCE.

Kristen Stout, MSP **Poster #71**

SYNAPTIC VESICLE GLYCOPROTEIN 2C (SV2C) MODULATES DOPAMINE RELEASE IN THE VENTRAL STRIATUM AND PALLIDUM

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The synaptic vesicle glycoprotein 2C (SV2C) localizes almost exclusively to neurosecretory vesicles of the basal ganglia, with strongest expression in the ventral pallidum. We generated SV2C knockout (SV2C-KO) mice to investigate the role the protein plays in modulation of dopamine release. Genetic ablation of SV2C reduces dopamine release in the ventral striatum and ventral pallidum. Behaviors associated with dopamine release were interrogated, including conditioned place preference, locomotor activity, sucrose preference, marble burying, forced swim test, and tail suspension. The mechanism by which SV2C augments vesicular dopamine release was interrogated using site-directed mutagenesis of glycosylation sites, as luminal sugar moieties are thought to stabilize neurotransmitter loading. Additionally, stimulus trains were used to investigate recruitment of vesicles from the reserve pool. Given its discrete expression, importance in dopamine neurotransmission, and contribution to reward behavior, SV2C is a promising target for the development of novel therapeutics for addiction treatment.

Thayer King, IMP **Poster #73**

EPHA2 IS REQUIRED FOR THE DEVELOPMENT OF EXPERIMENTAL CEREBRAL MALARIA

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Cerebral malaria is a severe complication of *Plasmodium falciparum* infection, yet the etiology of the disease remains unclear. In the well-established *Plasmodium berghei* ANKA (PbANKA) mouse model of experimental cerebral malaria (ECM), sequestration of parasitized red blood cells (pRBCs) and $CD8^+$ T cells in the brain microvasculature is required for ECM development. However, our understanding of the receptors involved in $CD8^+$ T cell retention in the brain during ECM is incomplete. We hypothesize that EphA2, a receptor tyrosine kinase, is involved in sequestering $CD8^+$ T cells in the brain during ECM through interactions with membrane-bound ephrin-A ligands on $CD8^+$ T cells. Using the PbANKA ECM model, we observe EphA2 upregulation in the brains of mice six days post-infection at ECM onset. Ephrin-A ligands are also upregulated on splenic T cells in PbANKA-infected mice three days post-infection prior to potential trafficking to the brain. Importantly, $EphA2^{-/-}$ mice show significant protection from ECM compared to $EphA2^{+/+}$ mice. Similar numbers of pRBCs accumulate in the brains of $EphA2^{-/-}$ and $EphA2^{+/+}$ mice, but $EphA2^{-/-}$ mice have less $CD8^+$ T cells in the brain six days post-infection correlating with improved survival. Our data demonstrates a novel role for EphA2 in the pathogenesis of ECM.

Dana Tedesco, IMP **Poster #75**

CD40L+ FOXP3+ CD4+ T CELLS PROMOTE ABERRANT IgG PRODUCTION DURING LIVER FIBROSIS

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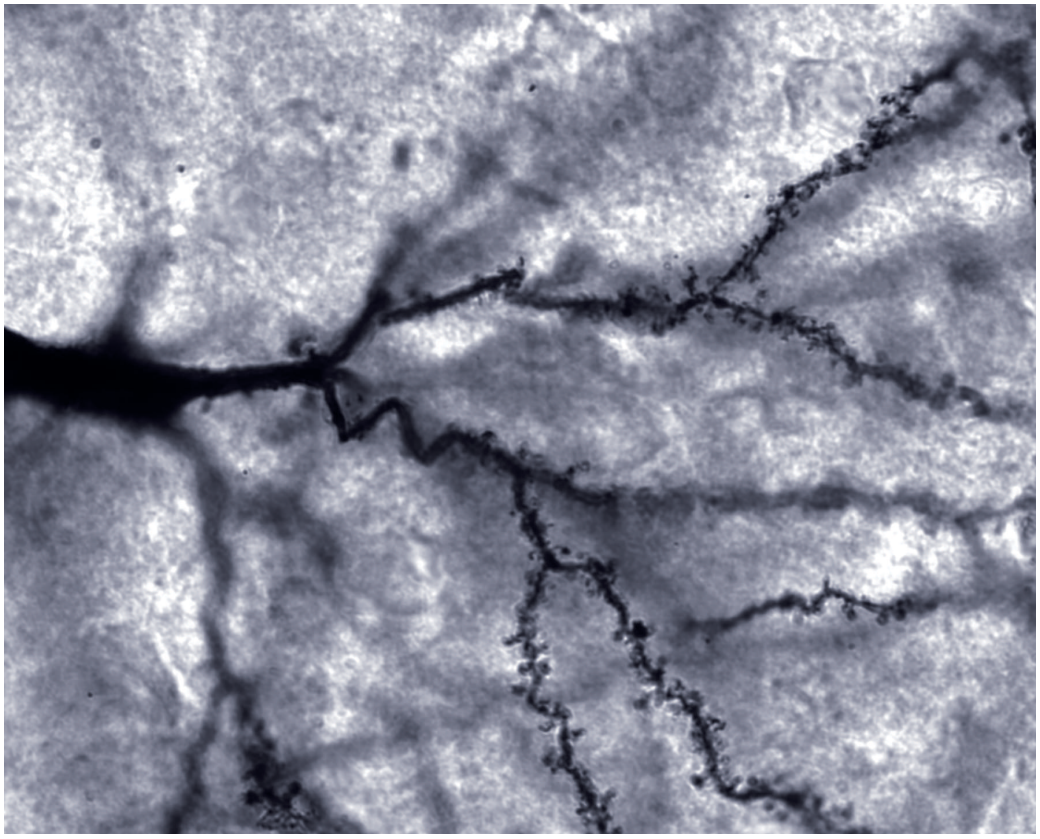
One of the most common causes of end-stage liver disease (ESLD) is hepatitis C (HCV) infection; the only therapeutic is a liver transplant. Extra-hepatic manifestations of ESLD include antibody-mediated autoimmunity; an indication of B cell and CD4 T cell dysfunction. We investigated the role of CD4+ T cells in the IgG-mediated systemic manifestations of liver fibrosis using antibody-mediated CD4+ T cell depletion in the carbon-tetrachloride (CCl_4) mouse model of liver fibrosis. Fibrotic animals exhibited elevated serum IgG, detectable ANA titer and spontaneous IgG production from liver B cells; this was not evident in CD4 depleted CCl_4 treated animals despite comparable indicators of fibrosis. To our surprise we found a fibrotic liver-specific accumulation of Foxp3+ CD4+ T cells that did not reduce B cell activity, yet effectively suppressed CD8+ T cell proliferation. Phenotypic analysis identified CD40L expression exclusively on a subset of fibrotic-liver Foxp3+CD4+ T cells which consequently permitted B cell activation *in vitro*. Fibrotic liver Foxp3+CD4+ T cells lacking CD40L suppressed B cells comparably to non-fibrotic and splenic controls. To investigate the relevance of this finding in human disease, we performed a parallel analysis in peripheral blood and livers of both HCV+ patients and non-fibrotic controls. We found a liver-specific accumulation of Foxp3+ CD4+ T cells in livers explanted from HCV+ patients that strongly correlated with spontaneous IgG production from intrahepatic B cells. Furthermore, a phenotypically similar CD40L+ Foxp3+ CD4+ T cell population was found exclusively in livers of HCV patients, but not non-fibrotic controls or patient-matched peripheral blood. Taken together, our data suggest that conserved fibrosis-elicited alterations on the CD4+ T cell compartment are critical for IgG-mediated extra-hepatic manifestations of liver fibrosis.



3rd place – Jadiel Wasson, BCDB

A medium spiny neuron in the brain of an adult animal golgi-stained to highlight all of the projections and dendritic spines

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Again, thank you, and we look forward to seeing you next year as we convene again to make the symposium a signature event for all of us in GDBBS.