TEXAS SYMPOSIUM ON
CRITICAL TOPICS IN IMMUNOLOGY

FEBRUARY 17-18, 2023

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SCHEDULE OF EVENTS
Texas Symposium on Critical Topics in Immunology
Texas A&M Hotel & Conference Center
Century Ballroom

Friday, February 17, 2023

7:45am-8:20am	Registration and Breakfast
8:20am-8:30am	**Welcome** John R. August, Carl B. King Dean of Veterinary Medicine Texas A&M University
8:30am-9:15am	**Opening Keynote Address** Florencia McAllister, Associate Professor MD Anderson Cancer Center
Session I: Vaccine & Therapeutic Approaches to Infectious Diseases	
Session chairs: James Samuel and Yeon Choi	
9:15am-10:00am	**Keynote Speaker** Stefan H.E. Kaufmann, Head of Immunology Max Planck Institute for Infection Biology
10:00am-10:30am	Scott Weaver, Distinguished Chair in Human Infections & Immunity The University of Texas Medical Branch
10:30am-10:45am	*Break*
10:45am-11:15am	Carolyn Cannon, Associate Professor Texas A&M University
11:15am-12:00pm	**Keynote Speaker** Peter Hotez, Dean of the National School of Tropical Medicine Baylor College of Medicine
12:00pm-1:30pm	Regional Collaboration in Training & Research Lunch: Laurel, Oak, Ross rooms (2nd flr) General Lunch: Century Ballroom
Session II: Immune Response to Pathogens: Host-Pathogen Interaction	
Session chairs: Kristin Patrick and Sarguru Subash	
1:30pm-2:15pm	**Keynote Speaker** Alan Sher, Distinguished Investigator, Laboratory of Parasitic Diseases National Institutes of Health
2:15pm-2:45pm	James Musser, Chair Pathology & Genomic Medicine Houston Methodist Research Institute
2:45pm-3:00pm	*Break*
3:00pm-3:30pm	Alison Coady, Assistant Professor The University of Texas Medical Branch
3:30pm-4:00pm	Maria Giraldo, Assistant Professor The University of Texas Medical Branch
4:00pm-4:30pm	Robbie Watson, Associate Professor Texas A&M University
4:30pm-6:30pm	Happy Hour & Poster Session I
6:30pm	Dinner

Saturday, February 18, 2023

Session III: Systems Immunology	
Session chairs: Paul de Figueiredo & Garry Adams	
7:45am-8:25am	Registration & Breakfast
8:30am-9:15am	**Keynote speaker** Venkata Vamsee Aditya Mallajosyula, Basic Life Research Scientist Stanford University
9:15am-9:45am	James Cai, Associate Professor Texas A&M University
9:45am-10:00am	*Break*
10:00am-10:30am	Vicky Yao, Assistant Professor Rice University
10:30am-11:00am	Alex Walsh, Assistant Professor Texas A&M University
11:00am-11:30am	Hyun-Sung Lee, Assistant Professor Baylor College of Medicine
11:30am-12:30pm	Lunch
12:30pm-1:30pm	Poster Session II
Session IV: Inflammatory Diseases including Cancer	
Session chairs: Phillip West & Jessica Galloway-Pena	
1:30pm-2:15pm	**Keynote Speaker** Nan Yan, Professor University of Texas Southwestern Medical Center
2:15pm-2:45pm	Farida Sohrabji, Regents Professor & Head of Neuroscience & Experimental Therapeutics Texas A&M University
2:45pm-3:00pm	*Break*
3:00pm-3:30pm	Seyed Javad Moghaddam, Associate Professor MD Anderson Cancer Center
3:30pm-4:00pm	Jianxun Song, Professor Texas A&M University
4:00pm-4:30pm	Poster Awards Ceremony & Closing Remarks

Keynote Speaker

Florencia McAllister

Florencia McAllister is a physician-scientist with basic science training in Host Defense and Tumor Immunology which she acquired during her postdoctoral training at the University of Pittsburgh and Johns Hopkins University. She trained in the laboratory of Dr. Steven Leach with co-mentorship from Dr. Drew Pardoll, leaders in Pancreatic Cancer and Tumor Immunology, respectively. She completed 2 clinical fellowships at Johns Hopkins University: in Medical Oncology with focus on Gastrointestinal Medical Oncology and Clinical Pharmacology. She was recruited to MD Anderson in 2014 and in the past five years she has established a translational research program focused on further understanding the fundamental microenvironmental mechanisms that influence pancreatic tumor initiation and progression with the ultimate goal of discovering novel immunopreventive and immunotherapeutic approaches for this disease. She has participated in key discoveries on T cell immunobiology, including unraveling the epithelial IL-17 signaling pathway, the characterization of IL-17-secreting immune cells in the initiation and progression of premalignant pancreatic and colorectal lesions and the key role of inflammation and bacteria induced T cells responses in the initiation and promotion of pancreatic and colon cancer. Recently, her lab published in Cell a study that implicates the gut-tumor microbial axis in modulating the tumor microenvironment, using the powerful approach of human-into-mice fecal microbial transplantation (FMT). Furthermore, she has developed a clinical platform, including a gastrointestinal cancer microbiome repository and pancreatic cancer high risk cohort, which will be very relevant to further validate the microbiome preclinical work in a clinically relevant population. Finally, her lab is currently performing a clinical trial using FMT in patients with pancreatic cancer prior to their surgery. These clinical platforms will be very important for the studies proposed. Therefore, she has the expertise and training necessary to offer my complete support for the P01 program project application entitled “IBIS: Integrating BRCA and Inflammatory Signaling”.

Keynote Speaker

Stefan Kaufmann

Founding Director of Max Planck Institute for Infection Biology, Berlin. Director Emeritus at Max Planck Institute for Infection Biology, Berlin, and at Max Planck Institute for Interdisciplinary Sciences, Göttingen. Senior Professor at Charité University Medicine, Berlin. Faculty Fellow of Hagler Institute for Advanced Study at Texas A & M University, College Station, Texas. Doctor Honoris Causa from Aix-Marseille University. Honorary Professor of Universidad Peruana Cayetano Heredia, Lima, Peru; Guest Professor at Tongji University, School of Medicine, Shanghai, China; Visiting Professor at Peking Union Medical College, Beijing, China. Scientific interests: immunity to bacterial pathogens with emphasis on tuberculosis and rational design of vaccines and biosignatures. Developer of a tuberculosis vaccine in several phase III clinical efficacy trials. Former President and honorary member of German Society for Immunology; former President of European Federation of Immunological Societies (EFIS) and of International Union of Immunological Societies (IUIS). Chair of the Board of Schering Foundation. Member of the Scientific Advisory Board and member of the Council of Lindau Nobel Laureate Meetings. Co-Chair of the Scientific Committee of World Health Summit. Member of the Governance Board of the TuBerculosis Vaccine Initiative (TBVI). Numerous prestigious scientific awards, most recently Gagna A. & Ch. Van Heck Prize in 2018 from FNRS, Belgium, Ernst Hellmut Vits Prize in 2022 from Universitätsgesellschaft Münster, Germany. Coordinator of several international and interdisciplinary projects, e.g. Grand Challenge 6 of the Bill and Melinda Gates Foundation from 2005 to 2016. > 900 publications mostly in high-ranking journals with > 90,000 citations, Highly Cited Researcher (immunology) 1981-1999 according to Thomson Institute for Scientific Information, Highly Cited Researcher (crossfield) 2020 according to Clarivate – Web of Science; amongst the 0.01% most cited scientists of ca. 7 million scientists in 22 major scientific fields globally (PLoS Biol 17(8): e3000384, 2019); h-index of >150 (Google Scholar)/ > 110 (Clarivate – Web of Science/ISI),). Editor or member of editorial boards of numerous international scientific journals; member of numerous professional societies and academies including American Academy of Microbiology, Berlin–Brandenburg Academy of Sciences and Humanities, German National Academy of Sciences Leopoldina, World Innovation Foundation and European Molecular Biology Organization (EMBO).

Keynote Speaker

Peter J. Hotez

Peter J. Hotez, M.D., Ph.D. is Dean of the National School of Tropical Medicine and Professor of Pediatrics and Molecular Virology & Microbiology at Baylor College of Medicine where he is also the Co-director of the Texas Children's Center for Vaccine Development (CVD) and Texas Children's Hospital Endowed Chair of Tropical Pediatrics**.** He is also University Professor at Baylor University, Fellow in Disease and Poverty at the James A Baker III Institute for Public Policy, Senior Fellow at the Scowcroft Institute of International Affairs at Texas A&M University, Faculty Fellow with the Hagler Institute for Advanced Studies at Texas A&M University, and Health Policy Scholar in the Baylor Center for Medical Ethics and Health Policy.

Dr. Hotez is an internationally recognized physician-scientist in neglected tropical diseases and vaccine development. As co-director of the Texas Children's CVD, he leads a team and product development partnership for developing new vaccines for hookworm infection, schistosomiasis, leishmaniasis, Chagas disease, and SARS/MERS/SARS-2 coronavirus, diseases affecting hundreds of millions of children and adults worldwide, while championing access to vaccines globally and in the United States. In December 2021, Dr. Hotez led efforts at the Texas Children's Center for Vaccine Development to develop a low-cost recombinant protein COVID vaccine for global health, resulting in emergency use authorization in India. He obtained his undergraduate degree in molecular biophysics from Yale University in 1980 (*phi beta kappa*), followed by a Ph.D. degree in biochemistry from Rockefeller University in 1986, and an M.D. from Weil Cornell Medical College in 1987. Dr. Hotez has authored more than 600 original papers and is the author of five single-author books, including *Forgotten People, Forgotten Diseases* (ASM Press); *Blue Marble Health: An Innovative Plan to Fight Diseases of the Poor amid Wealth* (Johns Hopkins University Press); *Vaccines Did Not Cause Rachel's Autism* (Johns Hopkins University Press); and *Preventing the Next Pandemic: Vaccine Diplomacy in a Time of Anti-science* (Johns Hopkins University Press). Dr. Hotez served previously as President of the American Society of Tropical Medicine and Hygiene, and he is founding Editor-in-Chief of *PLoS Neglected Tropical Diseases*. In 2006 at the Clinton Global Initiative, he co-founded the Global Network for Neglected Tropical Diseases to provide access to essential medicines for hundreds of millions of people. He is an elected member of the National Academy of Medicine (Public Health Section) and the American Academy of Arts & Sciences (Public Policy Section). In 2014-16, he served in the Obama Administration as US Envoy, focusing on vaccine diplomacy initiatives between the US Government and countries in the Middle East and North Africa. In 2018, he was appointed by the US State Department to serve on the Board of Governors for the US Israel Binational Science Foundation and is frequently called upon frequently to testify before US Congress. He has served on infectious disease task forces for two consecutive Texas Governors. For these efforts in 2017 he was named by FORTUNE Magazine as one of the 34 most influential people in health care, while in 2018 he received the Sustained Leadership Award from Research! America. In 2022 Hotez and his colleague Dr. Maria Elena Bottazzi were nominated for the Nobel Peace Prize for "their work to develop and distribute a low-cost COVID-19 vaccine to people of the world without patent limitation." Most recently as both a vaccine scientist and autism parent, he has led national efforts to defend vaccines and to serve as an ardent champion of vaccines going up against a growing national "antivax" threat. In 2019, he received the Award for Leadership in Advocacy for Vaccines from the American Society of Tropical Medicine and Hygiene. In 2021 he was recognized by scientific leadership awards from the AAMC (Association of American Medical Colleges) and the AMA (American Medical Association), in addition to being recognized by the Anti-Defamation League with its annual Popkin Award for combating antisemitism. Dr. Hotez appears frequently on television (including BBC, CNN, Fox News, and MSNBC), radio, and in newspaper interviews (including the New York Times, USA Today, Washington Post, and Wall Street Journal).

Keynote Speaker

Alan Sher

Alan Sher received his Ph.D from the University of California, San Diego working at the Salk Institute for Biological Studies and did his postdoctoral training at the National Institute for Medical Research in Mill Hill, London. After serving as an Assistant Professor at Harvard Medical School, he joined the National Institute for Allergy and Infectious Diseases in 1980 where he later became Chief of the Laboratory of Parasitic Diseases and was promoted to NIH Distinguished Investigator. Dr. Sher's research has focused on defining mechanisms of host resistance to human parasitic, mycobacterial and viral pathogens as well as the pathways which regulate the immune response to these important disease agents. This work has involved studies in experimental models as well as clinical research in disease endemic countries. His group has produced over 25 graduates who have gone on to run their own independent laboratories and/or departments. Dr. Sher is an elected member of the American Association for the Advancement of Science, American Academy of Microbiology, and Brazilian Academy of Science. He is the recipient of the NIH Director's Mentoring Award, the Bonazinga Award of the Society for Leukocyte Biology, the Bailey K. Ashford Medal of the American Society of Tropical Medicine and Hygiene, the BioLegend William E. Paul Award from the International Cytokine and Interferon Society and is an emeritus editor of the Journal of Experimental Medicine.

Keynote Speaker

Vamsee Mallajosyula

Dr. Vamsee Mallajosyula has made important contributions to our understanding of anti-viral immunity throughout his research career. During his PhD, he worked on an extremely challenging project, namely that of stabilizing the influenza hemagglutinin stem to elicit broad protection. That work is relevant to the development of universal flu vaccines. The designed immunogens were also the first to show significant cross-group protection. The work is subject of a patent application which has since been granted in multiple countries including USA and is in phase-I clinical trials. He received the B.H. Iyer medal for best thesis and was recognized with the INSA Medal for Young Scientists awarded by the Indian National Science Academy, Dept. of Science and Technology, Govt. of India.

During his postdoctoral work in Prof. Mark M Davis's group at Stanford University, Vamsee has successfully led projects studying the immune response in humans after vaccination and viral infections, specifically influenza and more recently SARS-CoV-2, both of which are of global importance. One of these accomplishments is the development of a novel peptide-MHC multimer platform (the 12-mer 'spheromer') to label specific T cells with increased sensitivity and specificity in comparison to existing technologies. More importantly, using the spheromer he unambiguously demonstrated an association between disease severity and cross-reactive T cell responses to seasonal coronaviruses. Subsequently, he also applied this technology to deeply profile T cells that arise in SARS-CoV-2 mRNA vaccine responses. He also continues to work on developing a more effective influenza vaccine and testing the idea of hemagglutinin fusions in a human tonsil organoid system. He has received grants from the Human Systems Immunology Pilot Project funded by the Bill & Melinda Gates Foundation to continue his promising research.

Keynote Speaker

Nan Yan

Nan Yan majored in biological sciences at Fudan University in Shanghai, China, but then moved to the U.S. in 2000 to pursue his PhD in the laboratory of Dr. Paul Macdonald at the University of Texas (UT) at Austin. His thesis focused on the translational regulation of mRNAs during *Drosophila* embryo development. He swapped flies for mammals as well as the topic for his postdoc and joined Dr. Judy Lieberman's laboratory at Harvard Medical School in 2006, where he investigated HIV-1 host–pathogen interactions. Nan set up his laboratory in 2011 at UT Southwestern Medical Center and continued in the immunology field but tilted his research toward the function of the innate immune system in monogenic rare diseases. His lab studies mechanisms of microbial immune evasion, autoimmune and autoinflammatory diseases including neuroinflammatory diseases with particular interest in inborn errors of innate immunity, including those that lead to rare neurodegenerative diseases.

TEXAS A&M UNIVERSITY

POSTER PRESENTATIONS
Texas Symposium on Critical Topics in Immunology

Session I | Friday, February 17 | 4:30-6:30 PM
Vaccine & Therapeutic Approaches to Infectious Diseases | Immune Response to Pathogens: Host-Pathogen Interaction

#	Name	Title
1	Aditi, Aditi	Generation of reporter cell lines for computational modelling of innate immune signaling
2	Browning, Sarah*	HIV and Mycobacterium tuberculosis co-infection impairs C-type lectin signaling pathways
3	Choi, Yeon	Combined gB (humoral) and IE1 (cell-mediated) vaccine strategies are sufficient for high efficacy cross strain protection against congenital cytomegalovirus
4	Dsilva, Ajai*	Virus-induced long noncoding RNAs regulate response to SARS-CoV-2 infection
5	Farris, Lauren*	Borrelia burgdorferi engages mammalian type I interferon responses via the cGAS-STING pathway
6	Gaber, Alhussien*	The antigenic variation system of Borrelia recurrentis, the agent of the louse borne relapsing fever
7	George, Iris*	Relative fitness of asymptomatic bacteriuria and uropathogenic E. coli Strains in urine.
8	Gonzales, Casey*	Germinal center collapse and attenuated B cell activation during severe Orientia tsutsugamushi infection in mice
9	Lamichhane, Prem	Effect of cellular signaling inhibition on stress granule assembly
10	Legere, Rebecca*	mRNA-Encoded Anti-VapA monoclonal IgG1 for passive immunization of foals against Rhodococcus equi
11	Martinez, Yazmin*	Helper T cell population bias following tuberculosis chemotherapy identifies opportunities for therapeutic vaccination to prevent relapse
12	McGregor, Alistair	Cytomegalovirus infection of guinea pigs is highly dependent upon viral pp65 for innate immune evasion counteracting IFI16/cGAS-STING pathway post-endocytic entry of epithelial, endothelial and placental trophoblast cells
13	Mishra, Abhishek*	BCG vaccine packaged human mesenchymal stem cells reprogram naive human macrophages into pro-inflammatory M1-macrophages and strengthen antimycobacterial immune responses in humanized mice.
14	Neumann, Natanel*	Examining a novel mouse model of Lyme neuroborreliosis
15	Paez, Reina*	A role for macrophage plasticity and activation in the differential expression of murine macrophage galactose-type lectin (MGL) orthologs
16	Palani, Sunil*	BALB/c and C57BL/6 mice differ in susceptibility to pneumococcal pneumonia after mild influenza A virus infection
17	Ross, Alyssa	Siderophore-linked microcin role in intraspecies competition
18	Shahinfar, Sheeva*	Eosinophilic annular erythema-like reaction to mRNA COVID-19 vaccine
19	Silveira, Bibiana*	Enteral Rhodococcus equi at birth induces trained immunity and protects foals against intrabronchial challenge
20	Teve, Michael*	Fatty acid homeostasis contributes survival of uropathogenic E. coli during copper toxicity
21	Thomas, Benjamin*	Novel protein DNA circuit for the detection of Shiga toxin
22	Torres, Manuel*	Macrophage glucocorticoid conversion influences the immune response in Diabetes-Tuberculosis comorbidity experimental model
23	VanPortfliet, Jordyn*	Impaired antibacterial innate immunity and elevated lung immunopathology in a model of PolG-related mitochondrial disease
24	Wager, Chrissy*	Mycobacterium tuberculosis evades host defense by activating transcription factor CREB in human macrophages
25	Wang, Liqing*	NAC1 restrains T cell memory formation during viral infection
26	Woolley, Michael*	A PCR-free rapid protocol for one-pot construction of highly diverse genetic libraries
27	Xing, Yikun*	Immunization with dual-protein combination, SinH and pro-HlyA, protects against ExPEC caused sepsis and cystitis in the murine models

*Indicates trainee

TEXAS A&M UNIVERSITY

POSTER PRESENTATIONS

Texas Symposium on Critical Topics in Immunology

Session II | Saturday, February 18 | 12:30-1:30 PM

Systems Immunology | Inflammatory Diseases including Cancer

#	Name	Title
28	Beevers, Samantha*	The influence of biological sex and age on B-cell profiles following TLR activation
29	Chockalingam, Karuppiah	Chicken-derived anti-CD20 antibodies with potent B-cell depletion activity
30	Coleman, Aja*	The Mycobacterium tuberculosis secreted protein Rv1075c hijacks host histone methyltransferases to promote infection
31	Dende, Chaitanya	Regulation of tissue-resident intestinal macrophages by circadian and dietary cues
32	Hanson, Braden*	Copper dependent inhibition of uropathogenic E. coli by a novel small molecule
33	Hissen, Karina*	Development of a gene expression assay for immunity in hybrid striped bass
34	Lei, Yuanjiu*	ZBP1 sustains type I interferon responses downstream of mitochondrial genome instability
35	Ott, Jeannine*	Evolution of surrogate light chain in tetrapods and the relationship between lengths of CDR H3 and VpreB tails
36	Peng, Hao*	The critical role of eukaryotic elongation factor 2 kinase in CD4 T cells
37	Said, Damilola*	Dendritic cells limit Th1 responses through the release of CTLA-4+ extracellular vesicles
38	Schein, Catherine	Breaking the serotype barrier: PCPcon antigens stimulate broad spectrum protection against alphaviruses.
39	Skow, Loren	scRNAseq reveals differences in gene expression of thymocytes between sexes, among inbred strains of mice and in mice exposed to an xenobiotic estrogen in utero.
40	Pan, Sharon* Tadjali, Armand*	A clinical presentation of idiopathic CD4 lymphocytopenia
41	Tang, Shu*	Ablation of long noncoding RNA MALAT1 activates antioxidant pathway and alleviates sepsis in mice
42	Tat, Vivian*	Characterizing spatial and temporal host innate immune responses to SARS-CoV-1 and -2 infection in pathologically relevant human lung epithelial cells
43	Zhang, Yan	Coxiella burnetii virulent phase I and avirulent phase II bacteria differentially interaction with neutrophils

*Indicates trainee

Poster #1

Generation of reporter cell lines for computational modelling of innate immune signaling

Aditi Aditi[1]*, Prem P. Lamichhane[1]* and Parimal Samir[1]

[1] *Department of Microbiology and Immunology, The University of Texas Medical Branch, Galveston, TX 77555*

Innate immune response is the first line of defense against viral infections. A complex array of intracellular signaling pathways such as, stress response, innate immune and programmed cell death signaling, are activated in response to viral infection to mount an efficient host response. Extensive cross talk exists between these pathways. To study the connection between these signaling pathways and cellular phenotype, single-cell reporters can be used to probe signaling dynamics and generate computational models of innate immune response. Here, we report the generation of florescent signaling reporter cell lines based on kinase translocation reporters (KTR). KTR system was invented by the Markus Covert lab and is based on conversion of phosphorylation of a reporter into a nucleocytoplasmic translocation. These biosensors are shown to be rapid, specific and quantitative reporters for various kinases, including MAPKs. We have generated stable mammalian reporter cell lines for ERK, JNK, p38 and PKA kinases, and validated these using LPS mediated signaling. Our future studies will focus on quantifying signaling pathways and cellular phenotypes in response to viral infection and build computational models of innate immune signaling. This work will greatly expand our knowledge of host cell responses to viral infections and guide future therapeutics.

Poster #2

HIV and *Mycobacterium tuberculosis* co-infection impairs C-type lectin signaling pathways

Sarah Browning[1], Reina Paez[1], Mark Endsley[1], Kubra Naqvi[2], Matthew Huante[1], Sadhana Chauhan[1], Benjamin Gelman[3], and Janice Endsley[1]

[1] *Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX 77555.*
[2] *Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA.*
[3] *Department of Pathology, University of Texas Medical Branch, Galveston, TX 77555.*

Infection with *Mycobacterium tuberculosis* (Mtb) is a serious and potentially life-threatening condition, especially in people with HIV (PWH). According to the WHO, there were 10.6 million new tuberculosis (TB) cases in 2021, and 700,000 of those cases were among PWH. Macrophages are a host cell for both pathogens and play an important innate immune role in determining the outcome of disease. C-type lectin receptors (CLR) are pattern recognition receptors (PRR) abundantly expressed on the macrophage surface that orchestrate the innate immune response to microbial insults. We previously identified a role for the macrophage galactose-type lectin receptor (MGL) CLR in protection against TB. In subsequent studies, we observed suppression of MGL in tissues of HIV-infected decedents and in human macrophages following in vitro infection with HIV. In human THP-1 cells and a humanized mouse model, we observe that HIV infection markedly impairs activation of MGL by mycobacteria in comparison to other CLRs with defined roles in antimycobacterial immunity including DC-SIGN and Dectin 1. We further determine roles for TLR2/6 heterodimer, TLR4, and TGF-Î² signaling pathways for transcriptional regulation of MGL. Importantly, these pathways have roles in innate immunity and pathogenesis following Mtb or HIV infections. These findings advance our understanding of MGL regulation as part of the CLR repertoire, as well as its role in the response to Mtb and to Mtb/HIV co-infection.

Poster #3

Combined gB (humoral) and IE1 (cell-mediated) vaccine strategies are sufficient for high efficacy cross strain protection against congenital cytomegalovirus

K. Yeon Choi[1], Nadia El-Hamdi[1], Alistair McGregor[1]

[1] *Texas A&M University School of Medicine, Bryan, TX*

VapA-specific memory B-cells were isolated from whole blood of an adult horse hyperimmunized against *R. equi* using flow cytometry-assisted cell sorting. Custom-designed PCR primers were developed against the full (*i.e.*, constant and variable domains) equine IgG1 heavy chain (H) and light chains (L) for use with the 10x Genomics™ sequencing platform. Sequencing of the V(D)J segments of H and L DNA amplified from sorted B-cells identified a high-frequency clonotype of an equine IgG1 mAb. The H and L sequences of this IgG1 mAb were cloned into individual plasmid vectors and successfully expressed in HEK-293T cells. DNA clones for the transcription of IVT mRNA encoding the mAb against VapA were generated using a plasmid vector for a HIS-tagged L and FLAG-tagged H of the mAb. Constructs of IVT mRNA were prepared using modified N1-methylpseudouridine-5'-triphosphate substitution and a poly(A) tail. Transfection using polyethylenimine and lipid nanoparticle transfection agents resulted in expression of the mAb within primary cultures of equine bronchial fibroblasts and HEK-293T cells. Specific recognition of the expressed mAb to virulent *R. equi* was demonstrated by colony immunoblot assays. Based on these results, we plan to investigate *in vivo* aerosol delivery of the VapA-specific IgG1 mRNA to foals to provide protection at the site of infection. This platform holds great potential for translation to veterinary and human medical applications, especially for delivering near-immediate protection to the airways with neutralizing antibodies in neonates.

Poster #4

Virus-induced long noncoding RNAs regulate response to SARS-CoV-2 infection

Ajai Dsilva[1], Sahana Jayakumar[1], Viraj Kulkarni[2], Smita Kulkarni[1]

[1] *Host-pathogen interactions program, Texas Biomedical Research Institute, San Antonio 78227.*
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**Correspondence to VK vkulkarni@txbiomed.org; SK skulkarni@txbiomed.org*

SARS-CoV-2 infection triggers immune reprogramming through global transcriptomic changes that drive the development of Coronavirus disease 2019 (COVID-19). Although differential expression and functions of protein-coding transcripts have been extensively studied in SARS-CoV-2 infection, less than 2% of the human genome encodes protein-coding transcripts. The majority of the transcriptome consists of non-protein-coding RNAs (ncRNAs). Long noncoding RNAs (lncRNAs), which constitute a large proportion of the transcriptome, regulate the expression of protein-coding genes, impact immune cell functions and response to infections, and have prominent roles in health and disease. However, the impact of cellular lncRNAs on SARS-CoV-2 pathogenesis is poorly understood. Our studies will provide fundamental insights into the role of lncRNAs in SARS-CoV-2 replication and how they alter host immune responses. We hypothesized that SARS-CoV-2-induced lncRNAs are critical regulators of viral replication and immune response. To test our hypothesis, we identified lncRNAs with significant differential expression in SARS-CoV-2 infected vs. uninfected cells across two cell types (A549-hACE2 and Calu) from published transcriptome data. We silenced the expression of the top lncRNA Bre- AS1 (BA) in a human lung epithelial cell model (A549 cells stably expressing hACE2 and hTMPRSS2, A549) using lncRNA-specific ASO (lncsi) or negative control (NC) and compared viral replication in lncsi vs. NC cells. BA-silencing (BA-si) increased SARS-CoV-2 replication and inhibited the expression of antiviral interferon-stimulated genes (ISG). Phosphorylated form of a transcription factor, signal transducer and activator of transcription [pSTAT3 (Tyr 705)] forms suppressor complexes that inhibit ISG transcription. We further show that Bre-AS1 inhibits the phosphorylation of STAT3 and enhances ISG transcription. Our data show that cellular lncRNAs could play significant roles in immune response and viral propagation. Thus, unraveling the mechanisms of lncRNA-mediated regulation of virus replication and immune response may lead to identifying new, highly selective therapeutic targets.

Poster #5

Borrelia burgdorferi engages mammalian type I interferon responses via the cGAS-STING pathway

Lauren C. Farris[1], Sylvia Torres-Odio[1], L. Garry Adams[2], A. Phillip West[1], & Jenny A. Hyde[1]

[1] *Department of Microbial Pathogenesis & Immunology, College of Medicine, Texas A&M University, Bryan, TX*
[2] *Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX*

Borrelia burgdorferi, the etiologic agent of Lyme disease, is a spirochete that modulates numerous host pathways to cause a chronic, multi-system inflammatory disease in humans. *B. burgdorferi* infection can lead to Lyme carditis, neurologic complications, and arthritis, dependent upon the ability of specific borrelial strains to disseminate, invade, and drive inflammation. *B. burgdorferi* elicits type I interferon (IFN-I) responses in mammalian cells and tissues that are associated with the development of severe arthritis or other Lyme-related complications. However, the innate immune sensors and signaling pathways controlling IFN-I induction remain unclear. In this study, we examined whether intracellular nucleic acid sensing is required for the induction of IFN-I to *B. burgdorferi*. Using confocal microscopy, we show that *B. burgdorferi* associates with mouse and human cells in culture. We report that IFN-I responses in primary mouse macrophages and murine embryonic fibroblasts are significantly attenuated in the absence of the pattern recognition receptor cyclic GMP-AMP synthase (cGAS) or its adaptor Stimulator of Interferon Genes (STING), which function together to sense and respond to intracellular DNA. In vivo tracking of bioluminescent *B. burgdorferi* during infection of C57BL/6 wild-type, cGAS knockout, or STING deficient mice revealed similar dissemination kinetics and borrelial load. However, tibiotarsal joint pathology and inflammation were reduced in cGAS knockout compared to wild-type mice. Collectively, these results indicate that the cGAS-STING pathway is an innate immune sentinel of *B. burgdorferi* that plays a key role in the induction of mammalian IFN-I responses.

Poster #6

The antigenic variation system of *Borrelia recurrentis*, the agent of the louse borne relapsing fever

Alhussien Gaber[1], John C. Blazer[2], Dominique J. Wiener[1], Artem, S. Rogovskyy[1]

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Louse-borne relapsing fever (LBRF) has a public health importance in a number of developing countries with significant morbidity and mortality. LBRF is caused by the louse-transmitted spirochete, *Borrelia recurrentis* (*B. recurrentis*), which is considered a strictly human pathogen with no animal reservoir. The LBRF pathogen is highly understudied because of the lack of suitable animal models. Until recently, the only available models were non-human primates and immunodeficient mice. By using the Collaborative Resource (CC), we have identified the first immunocompetent mouse model (CC046) using *B. recurrentis* strain A17. In this model, however, strain A17 was able to establish only transient, 3-day-long spirochetemia with no culture-detectable spirochetemic relapses. Although LBRF patients may exhibit a single spirochetemia, multiple relapses have also been reported. The capacity of *B. recurrentis* to establish spirochetemic relapses is determined by a makeup of the functional antigenic variation system. The latter is composed of active expression sites encoding two antigenically variable surface proteins, variable large proteins (Vlp) and variable small proteins (Vsp), and a number of respective, highly homologous vlp and vsp pseudogenes. Recombination between the expressible sites and pseudogenes result in antigenic variation of Vlp and Vsp, which in turn allow spirochetes to evade anti-*B. recurrentis* antibodies. Previous sequencing data suggested that the number of vlp and vsp pseudogenes could significantly vary between *B. recurrentis* strains. It is thus hypothesized that those *B. recurrentis* strains that possess a higher number of intact vlp and vsp copies will develop relapses of spirochetemia in CC046 mice. To test the hypothesis, we have had several *B. recurrentis* strains whole-genome sequenced by PacBio; and performed an extensive infectivity study using CC046 mice. As a result, the data demonstrated that, two out of five *B. recurrentis* strains tested had the capacity to develop multiple relapses of spirochetemia over a 20-day period. The pending whole-genome sequencing data may show that the identified infectivity phenotypes will correlate with the extent of vlp and vsp pseudogene repertoires in the tested strains.

Poster #7

Relative fitness of asymptomatic bacteriuria and uropathogenic *E. coli* strains in urine.

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Urinary tract infection is one of the most common infections worldwide. The main causative agent of UTI is uropathogenic *E. coli* (UPEC). There is an immediate need for novel treatment strategies because of increasing incidence of antibiotic resistance among uropathogens. An asymptomatic *E. coli* strain, ABU 83972 (ABU), was isolated from a Swedish girl who carried it for 3 years. As an alternative method of treatment, *E. coli* 83972 has been successfully utilized to intentionally colonize people who are prone to UTIs. Since ABU lacks significant virulence factors, P and type 1 fimbriae, it does not cause UTI. ABU is thought to suppress the colonization of UPEC by growing better in urine. However, the mechanisms behind the competition and growth repression by ABU are unclear and is the subject of this investigation. Here, the growth characteristics of the ABU were studied individually, and with those of other UPEC strains (CFT073 and UTI89) in LB medium and human urine. Each *E. coli* culture showed a similar growth curve when inoculated individually in LB media and human urine. Next, we performed competition experiments where ABU and UPEC strains were inoculated at a 1:1 ratio so that each strain represented 50% of the entire population. CFUs were enumerated at different time points to calculate relative abundance. 70-80% of the total population starting as early as 4 hours of incubation was ABU. Our results indicated that ABU significantly outcompeted CFT073 or UTI89 in human urine up to 96 hours of incubation. A similar pattern of competitive advantage for ABU against UPEC was observed when fresh human urine was added at 50 ï�l/minute flow rate to mimic the urine flow from kidney to bladder. ABU started to dominate the population reaching ~70% by 4 hours of incubation. On the contrary, UPEC grew better than the ABU strain in LB medium. Our findings highlight the importance of the nutrients found in the human urine for the growth of the ABU strain. Ongoing studies are aimed at detecting the components of human urine that augment the growth of ABU, and developing a mouse model of co-colonization with ABU and UPEC. In summary, our work establishes that ABU is better adapted for growth in human urine than UPEC.

Poster #8

Germinal center collapse and attenuated B cell activation during severe *Orientia tsutsugamushi* infection in mice

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Scrub typhus is a poorly studied but life-threatening disease caused by the intracellular bacterium *Orientia tsutsugamushi* (Ot). Cellular and humoral immunity in Ot-infected patients is not long-lasting, waning as early as one-year post-infection; however, its underlying mechanisms remain unclear. To date, no studies have examined germinal center (GC) or B cell responses in Ot-infected humans or experimental animals. This study was aimed at evaluating humoral immune responses at acute stages of severe Ot infection and possible mechanisms underlying B cell dysfunction. Following inoculation with Ot Karp, a clinically dominant strain known to cause lethal infection in C57BL/6 mice, we measured antigen-specific antibody titers, revealing IgG2c as the dominant isotype induced by infection. Splenic GC responses were evaluated by immunohistology, co-staining for B cells (B220), T cells (CD3), and GCs (GL-7). Organized GCs were evident at day 4 post-infection (D4), but they were nearly absent at D8, accompanied by scattered T cells throughout splenic tissues. Flow cytometry revealed comparable numbers of GC B cells and T follicular helper (Tfh) cells at D4 and D8, indicating that GC collapse was not due to excessive death of these cell subtypes at D8. B cell RNAseq analysis revealed significant differences in expression of genes associated with B cell adhesion (S1PR1, ICAM1, GPR183) and co-stimulation (CD40, ICOS-L, SLAMF1) at D8 versus D4. The significant downregulation of S1PR2 (a GC-specific adhesion gene) was most evident at D8, correlating with disrupted GC formation. Signaling pathway analysis uncovered downregulation of 71% of B cell activation genes at D8, suggesting a profound attenuation of B cell activation during severe infection. This is the first study showing the disruption of B/T cell microenvironment and dysregulation of B cell responses during Ot infection, which may help understand the transient immunity associated with scrub typhus.

Poster #9

Effect of cellular signaling inhibition on stress granule assembly

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Stress granules (SGs) are membraneless cytoplasmic compartment of ribonucleoproteins formed during cellular stress. SGs have been implicated in modulating host response to viral infections. The innate immune signaling was recently reported to inhibit SG assembly. In this study, we performed a targeted small molecule screen to identify modulators of SG assembly. We treated A549 cells, an alveolar epithelial cell line with the endoplasmic reticulum stressor thapsigargin with or without treatment with several kinase inhibitors at different doses. We have identified several kinase inhibitors that modulate SG assembly. Future research aims at elucidating the mechanistic basis of SG modulation by these kinase inhibitors and exploring their role in host response to viral infection for assessing their therapeutic potential.

Poster #10

mRNA-encoded anti-VapA monoclonal IgG1 for passive immunization of foals against *Rhodococcus equi*

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Pneumonia caused by *Rhodococcus equi*, a facultative intracellular pathogen, is an important cause of disease and death in newborn foals. Neonatal foals are most susceptible to infection because their naïve and immature immune responses fail to protect against inhaled *R. equi* which is ubiquitous in their environment. Virulent strains of *R. equi* express the virulence-associated protein A (VapA) that is necessary to cause disease in foals. Passive immunization by transfusion of hyperimmune plasma (HIP) protects foals against *R. equi* pneumonia, indicating a protective role for antibodies against *R. equi*; potency of commercial *R. equi* HIP is assessed by anti-VapA titers. Transfusion of HIP, however, is expensive, labor-intensive, and carries risks for foals including transfusion reactions and circulatory overload. Moreover, HIP products vary markedly in anti-VapA activity levels. Thus, our objective was to generate mRNA encoding an equine monoclonal antibody (mAb) of the IgG1 subisotype against VapA for passive immunization of neonatal foals.

Poster #11

Helper T cell population bias following tuberculosis chemotherapy identifies opportunities for therapeutic vaccination to prevent relapse

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700,000 people suffer tuberculosis (TB) recurrence annually following treatment and apparent clinical cure. Recurrence within one year of treatment is primarily due to relapse, an outcome associated with acquired drug resistance, mortality, and other poor clinical outcomes. Therapeutic vaccines have promise as adjunctive treatment for tuberculosis (TB) or as preventives against TB relapse. The T helper (Th) populations play critical roles in immunity to TB. The relative role and expansion of activated Th, and the balance with regulatory Th (e.g. IL-10+), populations, is incompletely understood in the context of relapse. Here, C57BL6J mice were infected via aerosol with Mtb-Beijing in a paucibacillary mouse model of post-drug TB relapse. The Th17 (IL-17+), Th1 (IFN-g+), Th2 (IL-4+), Treg-like (IL-10+), and Th22 (IL-22+) populations were assessed in lungs, spleen and blood, during active TB, at the end of anti-TB-drug treatment, and at TB relapse. Bacterial burden and cytokine secretion was further assessed at these different phases of infection and treatment. Active Mtb infection promoted expansion of Th1, Th2, Th17, and Th22 cells and significantly increased IL-17A, IL-22, and IFN-g cytokines in the lung. Following drug therapy, Th2, Th17 and Th22 populations contracted and Th cells producing IL-10 expanded. In contrast, Th1 cells and IFN-g cytokine remained elevated after drug therapy. At relapse, a moderate re-expansion of Th1 and Th17 populations were observed in lung along with a detectable increase in soluble IFN-g. Interestingly, Th22 failed to re-expand. The dynamics and polyfunctionality of Th populations further differed by tissue compartment and stage of infection. Interestingly, in a IL17RAKO, and an inducible Th17KO model, there were increased bacterial burdens at relapse compared with WT controls. These outcomes identify immune bias by Th subpopulations during TB relapse, especially Th17 and Th22, as candidate mechanisms for pathogenesis and targets for therapeutic vaccination.

Poster #12

Cytomegalovirus infection of guinea pigs is highly dependent upon viral pp65 for innate immune evasion counteracting IFI16/cGAS-STING pathway post-endocytic entry of epithelial, endothelial and placental trophoblast cells

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Cytomegalovirus is a betaherpesvirus and transplacental congenital cytomegalovirus is a leading cause of cognitive impairment and hearing loss in newborns. The guinea pig is the only small animal model for cCMV but requires species-specific guinea pig cytomegalovirus (GPCMV) as human CMV (HCMV) cannot be used directly in an animal model. GPCMV encodes functional homolog proteins to HCMV including viral glycoprotein complexes necessary for virus infection, which are important neutralizing antibody targets (e.g. gB). Our lab has established guinea pig cell lines for virus cell tropism studies including epithelial, endothelial and placental trophoblast cells. Both HCMV and GPCMV have two pathways of cellular entry: (1) direct fusion; (2) endocytic. PDGFRA cell receptor present on fibroblasts enables virus entry via direct fusion (using viral glycoprotein gB and gH trimer complex). Endocytic cell entry requires the viral glycoprotein pentamer complex (PC), gB and cell receptor NRP2. Guinea pig epithelial, endothelial and trophoblast cells lack PDGFRA and GPCMV entry is by endocytic infection pathway.

Similar to HCMV, GPCMV tegument layer contains a high percentage of a specific protein pp65 (GP83). A GPCMV GP83 knockout mutant virus (GP83dPC+) grew normally on fibroblast cells but was highly sensitive to IFN-I (100 IU/ml with 80% virus inhibition) compared to wild type virus (5% inhibition). On guinea pig endothelial, epithelial and trophoblast cells, GP83 knockout GPCMV was significantly impaired for infection (titer loss of up to 5 logs) and mutant virus was sensitive to both IFN-I and IFN-III. In an animal challenge experiment, GP83 mutant virus was highly impaired for dissemination to all target organs and viremia compared to wild type virus despite mutant encoding PC for entry into all non-fibroblast cells. Results indicated a role for GP83 in innate immune evasion, which was especially important for endocytic pathway of virus cell entry. In transient expression studies, immunoprecipitation assays demonstrated direct interaction of GP83 protein with guinea pig IFI16 and cGAS, suggesting a role in evasion of the cGAS-STING pathway in guinea pigs. Transient expression of GP83 on non-fibroblast cells restored mutant virus growth on epithelial, endothelial and trophoblast cells. Alternatively, GP83 mutant virus growth on non-fibroblast cells could be restored by degradation of IRF3 in cells expressing bovine viral diarrhea virus Npro protein, which specifically targeted IRF3 for degradation, restoring mutant GPCMV growth to wild type levels. Additionally, cellular ectopic expression of PDGFRA enabled direct fusion virus entry on epithelial and endothelial cells to restore mutant virus growth. Overall, results indicate that GP83 (pp65) has a novel important function specifically associated with endocytic pathway of cell entry in epithelial and endothelial cells related to innate immune evasion of IFI16/cGAS-STING pathway and IRF3 activation.

Poster #13

BCG vaccine packaged human mesenchymal stem cells reprogram naive human macrophages into pro-inflammatory M1-macrophages and strengthen antimycobacterial immune responses in humanized mice.

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Background: Although macrophages (MΦs) are a major niche for *Mycobacterium tuberculosis*, (Mtb), mesenchymal stem cells (MSCs) are an alternative niche for pathogen persistence. We found that human MSCs restrict Mtb through intrinsic autophagy and others showed that autologous MSCs had a therapeutic effect when transfused into MDR-TB patients. Because MSCs are widely used for immunotherapy of inflammation and cancer, we sought to determine the effects of BCG vaccine infected MSCs (BCG-MSCs) on human immune system.

Methods: Uninfected human MSCs or BCG Pasteur vaccine infected MSCs were cocultured with naïve MΦs separated by transwell chambers for 5 days. On days 0, 3 and 5, cocultured MΦs were subjected to RNAseq and single cell RNAseq 10X analysis followed by bactericidal functional analysis vs. MTB. Next, BCG packaged MSC or naïve MSCs were intravenously injected into humanized mice followed by RNAseq and CyTOF analysis of their immune system.

Results: *BCG-MSC both induce striking transcriptional changes in naïve MΦs after contactless coculture*: BCG-MSCs reprogramed naive human MΦs after 3-5 days coculture into a M1-MΦ phenotype and RNAseq-derived KEGG/GO analysis of programmed (Pro-MΦs) showed an elevated gene expression of modules regulating mycobacterial killing and antigen processing (*Phagosome-Lysosome-Antigen processing*, *NOD* and *TLR* signaling) compared to Pro-MΦs cocultured with naïve MSCs. 10X analysis validated expression of genes (e.g., *ATGs, cathepsins; HLA*) critical for antimycobacterial function and antigen processing. *Functional validation of* MΦ*-programming*: Pro-MΦs derived from BCG-MSC coculture showed enhanced mRNA expression for antimicrobial genes and killed Mtb *ex vivo*. Notably, humanized mice injected with BCG-MSC but not naïve MSCs upregulated genes regulating trained (TRI) and antimicrobial immunity (*ATGs, cathepsins*) in the lungs and spleens.

Discussion and conclusion: Although MSC transfusion suppresses MΦs and expands T-regs, autologous MSC transfusion improved clinical outcome in MDR-TB patients. Herein, we demonstrate that BCG 'packaged' MSCs positively skew naïve human MΦs into a hyper immunogenic M1-MΦ phenotype, increasing their anti-mycobacterial function paving the way for a stem cellular vaccine for treating TB. Indeed, BCG-MSC enhanced anti-tuberculosis immune signatures in humanized mice which suggested that a mesenchymal stem-cellular vaccine (MSTV) is feasible to treat MDR-TB patients. MSCs can cultured from donors in large scale and both autologous and heterologous transfusion is feasible. Persistence of Mtb within human MSCs is known, and we propose an intriguing hypothesis that low numbers of persisting Mtb in MSCs may be boosting immune system maintaining latent TB from reactivation. We conclude that MSCs are attractive vectors for immunotherapy. *Supported by NIH-NIAID RO1 AI-161015 (Jagannath, Khan, Endsley).*

Poster #14

Examining a novel mouse model of Lyme neuroborreliosis

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Lyme neuroborreliosis (LNB) is caused by some genospecies of Borreliella burgdorferi sensu lato complex (Bb), and is considered one of the highly understudied infectious diseases. The lack of mouse model of LNB has significantly hampered the LNB research. Until recently, nonhuman primates (NHPs) were the only model that demonstrated similarities to clinical manifestations of human LNB. However, issues of cost, animal availability, handling complexity, reagents availability, non-reproducible genetic backgrounds, and ethical concerns have significantly limited its use. In contrast to NHPs, all laboratory mouse strains tested did not exhibit clinical signs of LNB nor allowed Bb spirochetes or inflammatory cells to invade their neural tissues. Promisingly, most recent study demonstrated that Bb could colonize the dura mater of C3H mice, which resulted in meningitis. However, despite the Bb infection was associated with increased upregulation of inflammatory cytokines and interferon response in the both dura mater and brain parenchyma, the latter tissue was completely devoid of Bb spirochetes and infiltrating leukocytes. Moreover, the C3H mice did not exhibit any neurological signs of LNB. Recently, through the extensive use of the Collaborative Cross (CC) resource, we were able to identify one CC line, whose mice consistently developed significant inflammatory lesions in brains, spinal cords, and/or peripheral nerves upon Bb infection. Thus, in order to further examine our newly developed model of LNB, we have performed a long-term behavioral study. The latter included assessment of variable behavioral parameters of Bb-infected and uninfected (control) mice (i.e., total distance traveled, mouse gait movement, and anxiety-related behavior) utilizing the Noldus Ethovision and Mouse Specifics DigiGait. The statistical analysis demonstrated significant alterations in the mouse behavior due to Bb infection.

Poster #15

A role for macrophage plasticity and activation in the differential expression of murine macrophage galactose-type lectin (MGL) orthologs

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Macrophage plasticity and functional activation are critical to the broad array of macrophage-mediated functions that help determine disease outcomes. Macrophage galactose-type lectin (MGL), a type II transmembrane C-type lectin receptor (CLR) primarily expressed on immune cells of myeloid origin, has been shown to play an anti-inflammatory role in the innate response to some tumors and infectious agents. MGL has also been shown to play a role in mononuclear phagocyte recruitment, multinucleated giant cell formation, and synergistic signaling with Toll-like receptors (TLRs). Our lab recently demonstrated that MGL-1 contributes an antimicrobial and anti-inflammatory role in a murine model of experimental tuberculosis. We further observed that MGL-1 and MGL-2 expression by RAW 264.7 macrophages are similarly activated by whole mycobacteria, but differentially regulated by signaling through toll-like receptors (TLR) as well as cytokine and glucocorticoid receptors. We find that synthetic agonists and microbial ligands of TLR2, TLR4, and TLR7 positively regulate MGL-2 expression, suggesting responsiveness to a broad repertoire of antigens compared to MGL-1. Additionally, we find that activation of MGL-2 expression differs between ligands of multiple bacterial species, both pathogenic and non-pathogenic. Pathway inhibition experiments further show that MGL-2 upregulation by microbial stimuli occurs through MyD88-dependent TLR signaling. Although both MGL-1 and MGL-2 are both classically associated with alternatively activated macrophages, we find that MGL-2 expression is associated with both M1 and M2 polarized macrophages, while MGL-1 expression is restricted to M2. These results demonstrate a strong relationship between macrophage activation states and the differential activation of MGL signaling and indicate non-redundant roles of MGL-1 and MGL-2 in innate immunity of murine models of disease.

Poster #16

BALB/c and C57BL/6 mice differ in susceptibility to pneumococcal pneumonia after mild influenza A virus infection

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Influenza A virus (IAV) is a leading cause of respiratory infections in the United States. Post-influenza bacterial infections pose an increasingly severe threat to public health. Secondary bacterial pneumonia resulted in increased mortalities during the 1918 and 2009 flu pandemics. Streptococcus pneumoniae is the most common pathogen associated with secondary bacterial infection. It has been shown that IAV infection induces immune alterations in the host, which enables commensal bacteria, S. pneumoniae, to become a deadly pathogen. However, it remains unclear how host genetic factors contribute to this IAV/S. pneumoniae synergy. To that end, in the current study, we have compared BALB/c and C57BL/6 (B6) mice in their susceptibility to IAV/S. pneumoniae coinfection. IAV strain X31 (H3N2) infection alone causes mild disease in mice, and S. pneumoniae serotype 14 strain TJO983 (SPn14) is almost avirulent in mice. However, X31/SPn14 coinfection resulted in heightened bacterial outgrowth in B6 WT mice. Interestingly, in contrast to B6 mice, BALB/c mice can effectively clear the bacteria despite X31/SPn14 coinfection. Flow cytometric analysis of the airway immune cells showed increased numbers of inflammatory monocytes and neutrophils in B6 mice. Additionally, both BALB/c and B6 mice were able to clear the virus, suggesting that antiviral responses were not impaired. Similar findings were obtained in X31 and S. pneumoniae D39 (bacteremia strain) coinfection model. Bronchoalveolar lavage fluid (BALF) cytokine analysis revealed that coinfected B6 mice had significantly elevated levels of IFN-Î³ compared to BALB/c mice. Our ongoing studies focus on understanding how IFN-Î³ response contributes to the differences observed between the two strains of mice. Interpreting these genetic differences can help us better understand the risk factors associated with secondary bacterial pneumonia.

Poster #17

Siderophore-linked microcin role in intraspecies competition

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Urinary tract infections (UTI) are a major cause of antibiotic use and morbidity. Uropathogenic *Escherichia coli* (UPEC) is the primary cause of UTI. UPEC and other clinically significant uropathogens are part of the gut microbiome. Gut carriage is known to precede colonization of the urogenital tract and induction of UTI. How UPEC colonizes the human gut by competing with commensal *E. coli* is unclear. Better understanding of these intraspecies competition mechanisms is critical to develop non-antibiotic based approaches to prevent gut colonization. To answer this question, we screened a transposon mutant library of UPEC strain CFT073 for killing commensal *E. coli* strain (DH5Î±). Wild-type UPEC produces a clear zone of killing in the DH5Î± lawn. UPEC mutants that had smaller or no killing zones relative to parental strain were detected. We screened 4320 mutants and found 40 mutants with smaller zones than WT in the primary screen. In the secondary screen, 32 of these were confirmed to consistently have a smaller zone than the WT. These 32 mutants were sequenced to determine the Tn5 insertion site, 15 of the 32 mutants had insertion sites in the mch operon that encodes the production of a siderophore-linked microcin. Quantitative PCR assays revealed that Mch microcin production and killing of commensal stain by UPEC is regulated by Fur, the iron-sensing transcriptional regulator. Consistent with this observation, UPEC mutant lacking Fur was more effective at killing the commensal strain that the wild-type UPEC. Furthermore, commensal strains lacking select outer membrane ferrisideriphore importers were less susceptible to killing by wild-type UPEC. Ongoing work is evaluating the role of Mch in intraspecies bacterial competition in preclinical models of UPEC colonization. In summary, this work elucidates a key mechanism by which UPEC outcompete commensal *E. coli* in the gut.

Poster #18

Eosinophilic Annular Erythema-like reaction to mRNA COVID-19 vaccine

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Eosinophilic Annular Erythema (EAE) is a rare figurate dermatosis with tissue eosinophilia. It has a controversial connection to Wells Syndrome (WS) but is likely a separate entity due to clinical and histopathological differences. EAE typically presents with annular erythematous plaques with central clearing, and notably lacks the characteristic "flame figures" of WS, though there are reports of flame figures in EAE. We report a case of EAE-like lesions in the setting of mRNA COVID-19 vaccination. While local, distant, and generalized cutaneous reactions have been reported after vaccination, this is a unique report of an EAE-like eruption after COVID-19 vaccination.

A 61-year-old healthy woman presented with a 1-cm discrete erythematous annular pruritic plaque on the right lower leg, which progressed to numerous annular erythematous plaques with scalloped borders and central skin-colored to greenish hues, distributed over the arms, legs, and trunk.

The patient denied new medications, household members with similar eruptions, travel, or recent insect bites. The only notable feature in her history was covid vaccination within the prior 2 months, though she could not confirm the exact time course of her eruption as she felt the initial lesion may have gone unnoticed for weeks. She also noted flares after each dose or booster, of which she received both Pfizer and Moderna mRNA vaccination.

She was initially treated at an urgent care with topical antifungals for tinea corporis without improvement. She failed to improve with high-potency topical steroids. Laboratory studies revealed an absence of peripheral eosinophilia. Biopsies of her left forearm revealed perivascular infiltrates of lymphocytes and eosinophils with minimal epidermal change and flame figures. Her differential diagnoses included urticarial hypersensitivity reaction, arthropod reaction, Wells syndrome, erythema annulare centrifugum, EAE, and granuloma annulare. Her eruption cleared with oral corticosteroids but recurred after completion. She failed to improve with oral minocycline and declined therapy with hydroxychloroquine or immunosuppressive medication. She had partial clearance with 16 weeks of dupilumab.

Flame figures are seen in arthropod reaction, parasitic infections, hypereosinophilic syndromes, and Wells syndrome, among other diagnoses. There is continued discussion on whether EAE is a clinical variant of Wells syndrome. WS tends to present with prodromal pain and tender edematous lesions that appear cellulitic, peripheral blood eosinophilia, and flame figures on histopathology, EAE tends to present with a figurate erythema with central clearing and an absence of flame figures on pathology. Our patient's findings did not fit consistently into either category, though our leading diagnosis is EAE given the clinical appearance and case reports of flame figures along with lymphocytic and eosinophilic infiltration in EAE as previously noted. While the relationship between EAE and WS has been debated, it is possible that EAE as an annular or figurate erythema represents the broad clinical polymorphism of WS.

There have been reports of eosinophilia occurring in patients after COVID-19 vaccination. Although the entire mechanism of this response is unclear, it is postulated that mRNA vaccines stimulate a disproportionate amount of Th2 cytokines, which triggers hypereosinophilia and infiltration of eosinophils into tissues.

Poster #19

Enteral *Rhodococcus equi* at birth induces trained immunity and protects foals against intrabronchial challenge

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Rhodococcus equi is an intracellular pathogen that causes severe pneumonia in foals and immunocompromised people. Similar to other neonates, the immature adaptive immune system of foals renders them especially dependent on innate immunity. Our objective was to determine whether enteral live, virulent *R. equi* at birth induces trained immunity in foals and protects them against intrabronchial challenge at age 28 days. Foals were gavaged with saline (controls; n=6) or virulent *R. equi* (principals; n=5) at ages 2 and 4 days, and intrabronchially infected with *R. equi* at age 28 days. Blood was collected at ages 2 and 28 days for isolation of neutrophils and monocytes. RNA-sequencing (RNA-Seq) of neutrophils was performed and the differentially-expressed genes (DEG) used to select target genes for chromatin immunoprecipitation (ChIP)-qPCR. ChIP was performed with neutrophils and monocytes of foals for identification of regions enriched with H3K4me3 or H3K27me3 by qPCR for promoter regions of C1QA, CXCL10, CCL-8, IL-1α, IL-β, IL-1rn, IL-8, IL-32, RPL30, SECTM1, TNF-α, and VDR. H3K4me3 and H3K27me3 enrichment for each target gene was calculated using the percentage of input or ratio of percentages of input of H3K4me3 divided by H3K27me3. Data analysis was performed in R with significance set at $P < 0.05$ and included Fisher's exact test (clinical data), FastQC and Cutadapt (RNA-seq library quality), HISAT2 (mapping to equine genome), and DESeq2 (DEG RNA-Seq between groups). For CHIP-qPCR, the % of input and ratio were analyzed using linear-mixed effects regression with multiple pairwise comparisons made using the method of Tukey. Gavage with *R. equi* at ages 2 and 4 days significantly ($P < 0.05$) reduced the incidence of pneumonia (0%; 0/5) compared to control foals (67%; 4/6). We observed approximately 1,100 DEG in neutrophils of control and principal foals using bulk RNA-Seq, which included several trained immunity-related genes. In circulating monocytes from principal foals at age 28 days, we detected enrichment of H3K4me3 on promoter regions of C1QA, IL-1α, IL-8, SECTM1, TNF-α, and VDR. H3K4me3:H3K27me3 ratio was also increased in principals at age 28 days for C1QA, IL-1α, IL-β, IL-8, IL-32, and SECTM1. No significant epigenetic changes were detected on H3K4me3 in neutrophils, or H3K27me3 in either neutrophils or monocytes. To our knowledge, this is the first study to demonstrate that enteral administration of live bacteria (more specifically, *R. equi*) to newborn foals induced both expression of trained immunity-related genes in neutrophils and epigenetic changes on promoter region of trained immunity-related genes in monocytes. Our results indicate that trained immunity may be a mechanism protecting foals against pneumonia caused by intrabronchial challenge with *R. equi*.

Poster #20

Fatty acid homeostasis contributes survival of uropathogenic *E. coli* during copper toxicity

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Urinary tract infections (UTI) are highly prevalent infections with high levels of morbidity globally. Uropathogenic *Escherichia coli* (UPEC) is the leading cause of UTI. UPEC establishes a reservoir in the gut that leads to subsequent colonization of the urinary tract and induction of UTI. Studies from our team and colleagues have revealed an important role for cell envelope homeostasis and survival of *E. coli* during copper (Cu) toxicity. We have also demonstrated that Cu is a host effector mobilized to impede pathogen colonization during UTI. Effects of fatty acid metabolism on the virulence of enterohemorrhagic *E. coli* has been recently reported. Given the critical role of fatty acids in cell envelope homeostasis and virulence, we hypothesized that changes in fatty acid metabolism will affect survival of *E. coli* during Cu toxicity and attenuate its virulence in a mouse model of UTI. We screened a collection of mutants lacking various genes in fatty acid metabolism from the KEIO library for sensitivity and resistance to Cu. Mutants lacking fabR and fadR genes exhibited strikingly different Cu sensitivity compared to its parental strain. FadR is a dual transcriptional regulator of long chain fatty acid metabolism where it acts as a repressor of degradation and activator of biosynthesis. Inversely, FabR is a transcriptional repressor of the major fatty acid biosynthesis complex FabAB. We have now constructed and verified that UPEC fabR and fadR mutants also exhibit distinct Cu sensitivity profiles, compared to wild-type UPEC strain. Specifically, the mutant lacking fadR has increased resistance to Cu, whereas the fabR mutant has increased sensitivity to Cu. Ongoing work is assessing Cu accumulation and expression of Cu responsive genes in fabR and fadR mutants. In summary, our results demonstrate an important role for fatty acid metabolic pathways in combating Cu toxicity in UPEC.

Poster #21

Novel protein DNA circuit for the detection of Shiga toxin

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Shiga toxin producing *E. coli* (STEC) is the most common cause of bloody diarrhea, with over one million STEC infections occurring globally each year. Although early intervention has proven key in mitigating the risk of developing hemolytic uremic syndrome (HUS) due to STEC infection, the need for specialized lab tests (i.e., mass-spectrometry, ELISA, culture) significantly delays the STEC diagnosis and treatment. Building upon a panel of Designed Ankyrin Repeat Proteins (DARPins) with high affinity to Shiga toxin (Stx2), this study developed a novel protein-DNA circuit for rapid (<2 hour) and isothermal detection of Stx2.

Poster #22

Macrophage glucocorticoid conversion influences the immune response in Diabetes-Tuberculosis comorbidity experimental model

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During tuberculosis (TB) progress, macrophages (MQs) are considered hallmark cells for their plasticity and dual function throughout the infective process. MQs play oppositive functions supported by the tissue environment and disease progression. For instance, in the early immune response, alveolar MQs contribute negatively to bacterial spread. Nevertheless, upon activation by a Th1 response, these cells develop the avidity to induce protection. More in detail, MQs respond to environmental stimuli acquiring functional phenotypes in a process named polarization. This phenomenon is sustained by metabolic changes that allow MQs to maintain their effector roles.

Moreover, Type 2 diabetes in concomitance with TB (T2D/TB) is frequent and represents one of the most significant healthcare system challenges in developing countries. Several immunoendocrine abnormalities happen during the chronic phase of both diseases (T2D & TB), such as high extra-adrenal production of active glucocorticoids (GCs) caused by the activity of 11-β-hydroxysteroid dehydrogenase type 1 (11-βHSD1). 11-βHSD1 catalyzes the conversion of inactive cortisone to active cortisol or corticosterone in the lungs and liver, while the 11-β-hydroxysteroid dehydrogenase type 2 (11-βHSD2) has the opposite effect.

Active GCs have been related to insulin resistance and suppression of Th1 responses, which are deleterious factors in both T2D and TB. Specifically in advanced TB, the lungs show a higher concentration of cortisol, which partially suppresses inflammation but also inhibits protective immunity, allowing disease progression.

The anabolic adrenal hormone dehydroepiandrosterone (DHEA) exerts antagonistic effects on GC signaling in immune cells and metabolic tissues; however, due to anabolic effects can´t be used to treat immunoendocrine diseases. 16α-bromoepiandrosterone (BEA) is a water-miscible synthetic sterol related to DHEA that lacks an anabolic effect while amplifying the immune and metabolic properties with potential therapeutic uses.

More in detail, *in vitro* studies, demonstrated that blocking 11b-HSD1 with a specific inhibitor and BEA treatment increases the protection against *Mycobacterium tuberculosis* (M. tb) inducing the biosynthetic metabolism, macrophage polarization to M1 phenotype, and immune protective response compared with subtract-cofactor induced 11b-HSD1 and corticosterone.

Studies with animals showed that in the chronic phase of experimental tuberculosis, MQs increase active glucocorticoid conversion via 11b-HSD1 leading to a negative role in the course of infection. T2D/TB mice developed even more severe lung disease compared to the TB mice. After the treatment with BEA three times a week for two months, animals showed a decrease in the GC and 11-βHSD1 expression and an increase in 11-βHSD2 compared to untreated animals. These molecular effects of BEA were associated with a reduction in lung bacillary loads, and pneumonia.

In conclusion, we found a mechanism of immune response suppression in macrophages associated with immune susceptibility to tuberculosis. This immunoendocrine interaction is key to understanding the susceptibility to tuberculosis associated with diabetes. These results uphold the use of BEA as a promising effective therapy for T2D/TB co-morbidity or TB alone.

Poster #23

Impaired antibacterial innate immunity and elevated lung immunopathology in a model of PolG-related mitochondrial disease

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Mitochondrial diseases (MD) are a clinically heterogeneous group of disorders caused by respiratory chain dysfunction and metabolic failure. These diseases often result from pathogenic mutations in genes that function in oxidative phosphorylation or mitochondrial DNA (mtDNA) maintenance, are often debilitating, and have limited treatment options and no cures. Environmental exposures such as microbial infection appear to exacerbate the stepwise progression of MD. MD patients are also more susceptible to recurrent infections from opportunistic respiratory pathogens including Pseudomonas aeruginosa (PA). Infections can quickly spiral out of control, leading to sepsis and unrestrained inflammatory responses in MD patients. The underlying immune alterations in MD that impair antibacterial activity and enhance immunopathology are unclear, constituting a key gap in knowledge that complicates treatment and increases mortality in these patients. Thus, there is a critical need for mechanistic research to advance immune-focused therapies for MD. Mutations in mtDNA polymerase gamma (PolG) represent the most prevalent single-gene cause of MD. Previously published experiments using a faithful mouse model of PolG-related MD have uncovered novel immune alterations in PolG mutant animals, including elevated numbers of myeloid immune cells in the bone marrow and blood. Here, we report that PolG mutant mice exhibit increased myeloid cell infiltration and cytokine production in the lung after installation of bacterial lipopolysaccharide. This response is further exacerbated during installation of a low dose of live PA. Mechanistic studies also revealed widespread repression of the transcription factor nuclear erythroid 2-related factor 2 (Nrf2) in PolG mutant mice. Nrf2 orchestrates both antioxidant and anti-inflammatory responses, and stabilizing Nrf2 with the FDA-approved drug dimethyl fumarate (DMF) was effective at reducing inflammation in PolG macrophages exposed to live PA. Together, these finding suggest that loss of Nrf2 activity promotes myeloid cell hyperinflammation, lung immunopathology, and impaired antibacterial responses in PolG mutant mice and patients with PolG-related MD. Future work will characterize how reduced Nrf2 activity promotes hyperinflammatory innate immune responses, which contribute to acute lung injury that limits control of PA infection in PolG-related MD. This research will fundamentally advance our understanding of innate immune defects in a relevant model of MD and may pave the way for the development of innovative Nrf2-based therapies to mitigate infection-related immunopathology in PolG-related MD or other mitochondrial disorders.

Poster #24

Mycobacterium tuberculosis evades host defense by activating transcription factor CREB in human macrophages

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Mycobacterium tuberculosis (M.tb) uses host macrophages as an intracellular niche. A goal of our lab is to identify signaling pathways used by M.tb to modulate macrophage responses to promote bacterial survival and growth. Recently, we have determined that M.tb infection of human monocyte-derived macrophages rapidly induces activation of CREB, a transcription factor associated with anti-inflammatory signaling. However, CREBâ€™s role in human macrophage responses to M.tb is largely unknown. We have determined that inhibition of CREB signaling leads to decreased gene and protein expression of M.tb-induced immediate early genes COX2 and MCL-1, which are important for the immune response to M.tb. In contrast, CREB inhibition allowed for increased nuclear colocalization of NF-kB, suggesting increased activation of NF-kB signaling pathways. These early CREB-mediated signaling events predicted that CREB inhibition would lead to enhanced macrophage control of M.tb growth, which we indeed observed over days in culture. Decrease in bacterial growth corresponded with increased colocalization of M.tb with the late endosome/lysosome marker LAMP-1, indicating that M.tb activates CREB to limit phagolysosomal fusion. Interestingly, CREB inhibition also led to the subtle appearance of macrophage swelling which has been described for necroptosis. Consistent with this, we detected phosphorylation of RIPK1, RIPK3 and MLKL, hallmarks of necroptosis. However, we did not detect cell death at the time points tested. Recent literature has described non-necroptotic roles for pMLKL, including promotion of phagolysosomal fusion. Consistent with this observation, we found that the increased phagolysosomal fusion detected during CREB inhibition was dependent on RIPK1- and RIPK3-induced pMLKL, indicating that M.tb-induced CREB signaling blocks phagolysosomal fusion through inhibition of the necroptotic signaling pathway. Altogether, our data show that M.tb induces CREB activation in human macrophages early post-infection to induce an environment conducive to bacterial growth. Targeting certain aspects of CREB-induced signaling may lead to identification of novel targets for host-directed therapeutics to combat TB.

Poster #25

NAC1 restrains T cell memory formation during viral infection

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Nucleus accumbens-associated protein 1 (NAC1) is a transcriptional cofactor that has been extensively investigated in stem cell and cancer biology. Our recent studies indicate that NAC1 can modulate the functional activity of regulatory T cells and tumoral expression of NAC1 restrains antitumor immunity. However, little is known about its regulation of memory T cells. Furthermore, T cell memory is important for host to defend recurrent infections. Many factors have been reported to influence the T cell memory formation, such as transcriptional factor, co-stimulatory factor and metabolism status. Improving T cell memory will benefit for vaccine efficacy elongation.
In the current study, we analyzed both CD4+ and CD8+ T cells from wild type (WT) and NAC1-deficient (-/-) mice and used the vaccinia virus (VACV) as a tool to investigate the precise role and mechanism of NAC1 in controlling CD4+ and CD8+ T cell memory.
In vitro, we identified that NAC1 is essential for T cell metabolism, including both glycolysis and oxidative phosphorylation, positively modulating CD4+ and CD8+ T cell survival. Mechanically, loss of NAC1 caused defective autophagy and interrupted the AMPK-mTOR pathway in CD4+ T cells. NAC1-/- CD8+ T cells upregulate IRF4 stabilization.
In vivo, when i.p. challenged with VACV, NAC1-/- mice had improved VACV-specific CD4+ and CD8+ T cell formation at 35 days compared with WT mice. NAC1 acts as a suppressor of CD4+ T cell memory formation through the autophagy pathway. IRF4 seems to be involved in CD8+ T cell formation. Based on previous research, autophagy and IRF4 were proven to regulate T-cell memory formation. Targeting NAC1 may be adapted to promote memory CD4+ and CD8+ T cell development during viral infection.

Key Words: T cells; memory; cell metabolism; NAC1; immune regulation

Poster #26

A PCR-free rapid protocol for one-pot construction of highly diverse genetic libraries

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In vitro protein display methods can access extensive libraries (e.g., 10^12, 10^14) and play an increasingly important role in protein engineering. However, the preparation of large libraries remains a laborious and time-consuming process. Here we report an efficient one-pot ligation & elongation (L&E) method for sizeable synthetic library preparation free of any PCR amplification or purification steps. As a proof of concept, we constructed an ankyrin repeat protein templated synthetic library with 10^11 variants in 150 ÂµL volume. The entire process from the oligos to DNA template ready for transcription is linearly scalable and took merely 90 minutes. We believe this L&E method can significantly simplify the preparation of synthetic libraries and accelerate in vitro protein display experiments.

Poster #27

Immunization with dual-protein combination, SinH and pro-HlyA, protects against ExPEC caused sepsis and cystitis in the murine models

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Extraintestinal pathogenic Escherichia coli (ExPEC) is the leading cause of adult life-threatening sepsis and urinary tract infections (UTI). The emergence and spread of multidrug-resistant (MDR) ExPEC strains result in a considerable amount of treatment failure and hospitalization costs, and contribute to the spread of drug resistance amongst the human microbiome. Thus, an
effective vaccine against ExPEC would reduce morbidity and mortality and possibly decrease carriage in healthy or diseased populations. Hemolysin (HlyA) is a potent and ubiquitous cytolysin, which ExPEC strains expressing HlyA correlate with increased severity and dissemination of infection. Here, we evaluated the protective efficacy and immunogenicity of the
inactive protoxin of HlyA, pro-HlyA, and the Dual-Hit, the mixture of the pro-HlyA and previously reported invasin-like protein SinH. Immunization of a murine host with pro-HlyA or Dual-Hit elicited significant protection against multiple pandemic ExPEC sequence types strains, ST73 (CFT073) and ST95 (UTI89), in model of bacteremia. Both pro-HlyA or Dual-Hit immunization also provided significant protection against ExPEC colonization in the bladder in an acute UTI model. Immunized cohorts produced significantly higher levels of vaccine-specific serum and urinary IgG, findings consistent with mucosal protection. Collectively, these results demonstrate that single-protein pro-HlyA or double-protein combination of pro-HlyA and SinH, may constitute promising ExPEC phylogroup-specific and sequence-type effective vaccine targets that reduce E. coli colonization and virulence.

Poster #28

The Influence of Biological Sex and Age on B-Cell Profiles Following TLR Activation

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Background: Immunosenescence is a process resulting in dysfunction of the immune response with age. Immunosenescence affects the ability to resist and respond to infection and vaccines, and results in increased susceptibility to autoimmune diseases, degenerative diseases, and cancers. Biological sex also influences the immune system. Males tend to have a less robust adaptive immune response than females, with an increasing difference with age. Females have a higher incidence of autoimmune and autoinflammatory diseases, the incidence of which also increases with age. The most notable cellular changes in immunosenescence are changes in the adaptive immune response involving specific types of T-cells and B-cells. Accumulation of one subset of atypical B cells, termed age-associated B cells, is a key age-related change in B cell compartments. Characteristics of these age-associated B cells include the expression of 41BBL. Our work has demonstrated that inflammatory events, including infection and injury, cause an expansion of pro-inflammatory subsets of B cells, including CLIP+ B cells. CLIP, also known as Major Histocompatibility Complex Class II (MHC II)-associated invariant peptide, occupies the antigen-binding groove of both intracellular and, in some cases, cell surface MHC II molecules. How age and biological sex affect the expansion of cell surface CLIP+ and 41BBL+ B cells is unknown. It is possible that immunosenescence, and the accompanying expansion of age-associated inflammatory B cells, may be involved in the increased comorbidities and complications that are often experienced by the older population. The current study tested the hypothesis that in response to inflammatory challenge, age and sex would exacerbate the expansion of pro-inflammatory CLIP+ and/or 41BBL+ B cells.

Methods: Spleens from male and female wild type mice, 12 and 23 months of age, were collected and processed. Splenocytes were treated with CpG to stimulate an inflammatory response via TLR-9 activation and were incubated for 48 hours at 37â„ƒ. Following treatment, splenocytes were stained with CD90.2 (T-cells), CD19 (B-cells), 41BBL (an established marker for inflammatory B cells) and CLIP (a cleavage product of CD74), were then analyzed using flow cytometry.

Results: CpG treatment significantly upregulated the percent of CLIP+ B-cells in splenocytes in all age groups and both sexes. However, there was a significant effect of age, with older animals showing an elevated response to CpG treatment. Additionally, splenocytes from 12-month-old female mice exhibited a greater percentage of CLIP+ B cells compared to age-matched males. Conversely, splenocytes from 23-month-old female mice had a reduced frequency of CLIP+ B cells compared to age-matched male mice.

Conclusion: The results from this study show an increased B cell, CLIP+ and 41BBL+ B cell frequency in response to CpG. Age and sex appear to contribute to the effects of TLR activation. The data support the hypothesis that there is an age-dependent increased expansion of pro-inflammatory B cells that may contribute to the co-morbidities associated with age, potentially providing a novel therapeutic target for age-related inflammatory conditions. Future studies will further investigate age- and sex-related changes in immunity and how those changes may contribute to age associated increases in autoimmunity.

Poster #29

Chicken-derived anti-CD20 antibodies with potent B-cell depletion activity

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Antibodies (Abs) targeting the human B cell marker CD20 are able to specifically target B cells for depletion by engaging with immune effector functions and have played a key role in the treatment of B cell malignancies and autoimmune disorders for decades. Existing clinically used anti-CD20 Abs, including rituximab (RTX), remain only partially effective, often requiring extended treatment and/or combination with chemotherapy or other immunotherapies/targeted agents. We report four novel anti-human CD20 monoclonal Abs discovered from a phylogenetically distant species of chickens. The chicken-human chimeric antibodies exhibit at least 10-fold enhanced antibody-dependent cellular cytotoxicity (ADCC) and 4-8-fold stronger complement-dependent cytotoxicity (CDC) relative RTX. One of the Abs, AC1, can bind mouse CD20, indicating specificity for a novel CD20 epitope inaccessible to current (mouse-derived) anti-CD20 Abs. A humanized version of one Ab, hAC11-10, was created by complementarity-determining region (CDR) grafting into a human variable region framework. These Abs represent promising therapeutic candidates for improving upon current less-than-ideal clinical outcomes in lymphoid malignancies and autoimmune disorders and provide an arsenal of biologically relevant molecules for the development of next-generation CD20-mediated immunotherapies including bispecific T-cell engagers (BiTE), antibody drug conjugates (ADC) and chimeric antigen receptor- engineered T (CAR-T) cells.

Poster #30

The *Mycobacterium tuberculosis* secreted protein Rv1075c hijacks host histone methyltransferases to promote infection

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Mycobacterium tuberculosis (Mtb) remains the most infectious and deadly pathogen worldwide. Key to Mtb virulence are Mtb membrane-bound and secreted effector proteins that interact with host proteins to influence the host response to infection and promote Mtb survival. Rv1075c is an Mtb effector protein that traffics to the macrophage nucleus where it interacts with components of the SET1 histone methyltransferase complex. We hypothesize that Mtb expresses Rv1075c to promote a pro-bacterial innate immune gene expression program in infected macrophages. Consistent with this, ectopic expression of Rv1075c in macrophages elicits hyperinduction of Ifnb1, which is associated with Mtb pathogenesis in vivo.

Poster #31

Regulation of tissue-resident intestinal macrophages by circadian and dietary cues

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Intestinal Tim4+ CD4+ macrophages are a distinct macrophage subset that expresses Tim4, a receptor for phosphatidylserine on dying apoptotic cells. Unlike other macrophage subsets, they do not depend on blood monocytes for their turnover and instead are self-maintained in the small intestine. The signals responsible for the self-maintenance and function of Tim4+ CD4+ macrophages are unknown. We have discovered that maintenance of the gut resident Tim4+ CD4+ macrophage population depends on dietary vitamin A and its derivative retinoic acid (RA). Retinoic acid receptors (RARs), which direct RA-dependent transcription, were required to maintain Tim4+ CD4+ macrophages. Chemical blockade of RARsignaling and macrophage-specific genetic inactivation of RARs in mice further revealed that macrophage-intrinsic RARÎ± signaling was required for Timd4 expression and maintenance of Tim4+ CD4+ macrophages. Tim4+ CD4+ macrophages were involved in clearing dying epithelial cells under homeostatic conditions. In addition, Tim4+ CD4+ macrophages varied across the day-night cycle and thus exhibited circadian rhythmicity. Mechanistically, we found that the expression of RA biosynthetic enzymes was rhythmic, generating RA in a diurnal manner to drive Timd4 expression. Finally, RA biosynthetic enzymes and Timd4 expression depended on the macrophage-intrinsic clock. These findings indicate that circadian and dietary cues converge to regulate tissue-resident Tim4+ CD4+ macrophage maintenance and function in the mouse intestine. These findings may have potential implications for treating gastrointestinal diseases.

Poster #32

Copper dependent inhibition of uropathogenic *E. coli* by a novel small molecule

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Urinary tract infections (UTI) are a global health problem caused mostly by uropathogenic E. coli (UPEC) and is the second leading cause of prescribed antibiotics. Antimicrobial resistance in UPEC and other uropathogens is climbing at an alarming rate posing a threat to the current treatment practices. Recent findings have shown that copper is mobilized to the bladder as an innate host-immune response. We hypothesized that small molecules that increases the toxicity of physiologically-relevant concentrations of copper to UPEC would mitigate UTI. A small molecule library consisting of 51,098 unique molecules was screened against UPEC in the presence of copper and hits were triaged to a singular molecule of interest that acts in a bacteriostatic manner. This single molecule was assigned the name â€œE. coli Inhibitorâ€� or â€œECIN.â€� Inductively coupled plasma mass spectrometry revealed that UPEC treated with ECIN in combination with copper accumulated higher levels of cell-associated copper. Additionally, quantitative PCR analysis showed an upregulation major copper efflux gene in UPEC when treated with ECIN and copper. Together these results suggest that ECIN accentuates copper stress in UPEC. Further analysis of the combinatory treatment of ECIN and copper and its impact on the transcriptome revealed that UPEC activates stress response systems to restore envelope homeostasis and efflux mechanisms to decrease intracellular ECIN concentration. Future studies will be performed to evaluate the potential of ECIN to aid in the restoration of antibiotic sensitivity, the resistance potential against ECIN, obtain a better understanding the mechanism of action, and evaluation of therapeutic potential in a murine model of UTI. In summary, we have identified a novel small molecule that inhibits growth of UPEC in a copper-dependent manner.

Poster #33

Development of a gene expression assay for immunity in hybrid striped bass

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Objective

Freshwater aquaculture is growing in demand with consumers reducing the dependency of marine fisheries. The teleost fish, hybrid striped bass (HSB), a cross between striped bass (*Morone saxatilis*) and white bass (*M. chrysops*), have been introduced to combat oceanic overfishing and can tolerate the farm environment. However, the health of premium fish like HSB become important for sustainability goals, since the industry is constantly challenged with mortality due to pathogens. Because teleosts have both an innate and adaptive immune system, we can use them as a model to understand better the relationship between systemic immune response and its effects from amino acid supplementation to improve overall fish health. Immune proteins such as cytokines were measured through RNA expression levels in HSB spleens and were analyzed to determine the effects of immune response from amino acid supplementation, like glycine, compared with a non-supplemented diet. This will provide a better understanding of the effects of systemic immune response from the amino acid glycine, with the hope to overall improve fish health and production with this kind of supplementation.

Materials and Methods

HSB were housed at Texas A&M Aquaculture Facility, where they. Were either fed a control diet containing no amino acid supplementation or a 2% glycine-supplemented diet. Half of the fish from each group were treated with an immunostimulant by intraperitoneal (IP) injection of 0.1 ml of RPMI media containing 100 Î¼g of trinitrophenyl-lipopolysaccharide, or a control IP injection of 0.1mL of RPMI media. On the day of termination, the spleen and muscle were collected and immediately flash-frozen in liquid nitrogen and stored at -80C. RNA was extracted from the muscle and spleen, then cDNA was synthesized for quantitative real-time PCR to determine the relative expression of mRNAs for 5 innate immune proteins, and 4 adaptive immune proteins employing delta-delta-Cq.

Results

The relative gene expression analysis displays that the glycine-supplemented diet upregulated innate immune genes of interest approximately 2-fold and 5-fold for TNFÎ±, indicating a possible relationship between glycine and macrophage-produced cytokines. The glycine diet downregulated some adaptive immune genes, including TGFÎ² and IFN-Î³ less than 1-fold, but remarkably upregulated IgT over 50-folds. IgT is an antibody that teleost fish secrete within their mucosa. IgT is analogous to IgA in mammals, but the fold change was greater than expected, indicating that the glycine supplementation may impact mucosal immune response. The results suggest that there may be a relationship with systemic innate macrophage-produced cytokines upregulating the specific adaptive protein, IgT, in the mucosa.

Conclusions

This preliminary sets up the next experiment for further investigation on amino acid supplementation effects on the gut mucosa compared with other immune tissues. By utilizing a purified amino acid supplement instead of a non-crystalline amino acid, it will be the sole protein source in the diet. We hope to gather more mRNA expression data on HSB immune response systemically and within the intestinal mucosa to bridge the gap in the relationship between intestinal mucosa immunity and systemic immune response.

Poster #34

ZBP1 sustains type I interferon responses downstream of mitochondrial genome instability

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Mitochondrial DNA (mtDNA) is a potent agonist of the innate immune system; however, the exact immunostimulatory features of mtDNA and the kinetics of mtDNA detection by cytosolic nucleic acid sensors remain poorly defined. Here, we show that mitochondrial genome instability leads to Z-form mtDNA accumulation. Z-DNA Binding Protein 1 (ZBP1) stabilizes Z-form mtDNA and nucleates a cytosolic complex containing cGAS, RIPK1, and RIPK3 to sustain STAT1 phosphorylation and type I interferon (IFN-I) signaling. Increased mitochondrial Z-DNA, ZBP1 expression, and IFN-I responses are observed in cardiomyocytes after exposure to Doxorubicin, a first-line chemotherapeutic agent that induces frequent cardiotoxicity in cancer patients. Strikingly, mice lacking ZBP1 or IFN-I signaling are protected from Doxorubicin-induced cardiotoxicity. Our findings reveal ZBP1 as a cooperative partner for cGAS that sustains IFN-I responses to mitochondrial genome instability and highlight ZBP1 as a potential target in heart failure and other disorders where mtDNA stress contributes to interferon-related pathology.

Poster #35

Evolution of surrogate light chain in tetrapods and the relationship between lengths of CDR H3 and VpreB tails

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In the mammalian immune system, the surrogate light chain (SLC) shapes the antibody repertoire during B cell development by serving as a checkpoint for production of functional heavy chains (HC). Structural studies indicate that tail regions of VpreB contact and cover the third complementarity-determining region of the HC (CDR H3). However, some species, particularly bovines, have CDR H3 regions that may not be compatible with this HC-SLC interaction model. With immense structural and genetic diversity in antibody repertoires across species, we evaluated the genetic origins and sequence features of surrogate light chain components. We examined tetrapod genomes for evidence of conserved gene synteny to determine the evolutionary origin of VpreB1, VpreB2, and Î»5, as well as VpreB3 and pre-T cell receptor alpha (PTCRA) genes. We found the genes for the SLC components (VpreB1, VpreB2, and Î»5) only in eutherian mammals. However, genes for PTCRA occurred in all amniote groups and genes for VpreB3 occurred in all tetrapod groups, and these genes were highly conserved. Additionally, we found evidence of a new VpreB gene in non-mammalian tetrapods that is similar to the VpreB2 gene of eutherian mammals, suggesting VpreB2 may have appeared earlier in tetrapod evolution and thus, may be a precursor to traditional VpreB2 genes in higher vertebrates. Among eutherian mammals, sequence conservation between VpreB1 and VpreB2 was low for all groups except rabbits and rodents, where VpreB2 was nearly identical to VpreB1 and did not share conserved synteny with VpreB2 of other species. More likely, VpreB2 of rabbits and rodents represents a duplicated variant of VpreB1 and is distinct from the VpreB2 of other mammals. Therefore, rabbits and rodents have two variants of VpreB1 (VpreB1-1 and VpreB1-2) but no VpreB2. Sequence analysis of VpreB tail regions indicated differences in sequence content, charge, and length, and where repertoire data was available, tail length differences were correlated with CDR H3 length. Thus, we posit that SLC components co-evolved with antibody HC to accommodate the repertoire of particularly CDR H3 length and structure, and perhaps highly unusual HC (like ultralong HC of cattle) may bypass this developmental checkpoint altogether.

Poster #36

The critical role of eukaryotic elongation factor 2 kinase in CD4 T cells

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T cells are a primary source of inflammatory cytokines to mediate immune responses. Evidence shows that a particular subset of CD4 T cells, IL-17-secreting helper CD4 T cells (Th17 cells), play a vital role in inflammation, leading to autoimmune diseases. Eukaryotic Elongation Factor 2 Kinase (eEF2K) is a protein kinase that negatively modulates the elongation step of translation and further regulates vascular inflammatory responses and hypertension development. However, it is still unclear how eEF2K impacts CD4 T cells. Understanding T cell functions, such as T cell inflammation and metabolism, can significantly advance our knowledge toward the goal of improving health span. We revealed that eEF2K deficient mice displayed defects in CD4 T cell survival and increased inflammatory cytokine production. By analyzing CD4 T cells from the wildtype and eEF2K deficient mice, we discovered that eEF2K regulates mitochondrial activities, including oxidative phosphorylation, mitochondrial stress, and reactive oxygen species (ROS) production, which is related to inflammation. We found that STAT3, the major transcription factor of Th17 and a master regulator of mitochondria, is upregulated in CD4 T cells without eEF2K. These results suggest that eEF2K is essential in regulating STAT3 and CD4 T cell metabolism. Further investigations on the significance of eEF2K in diseased models, including T cell transfer colitis, will help understand the essential role of eEF2K in CD4 T cells and design novel strategies by targeting eEF2K-based immunotherapy in autoimmune diseases.
Key Words: CD4 T cells; eEF2K; T cell activation; metabolism; mice

Poster #37

Dendritic cells limit Th1 responses through the release of CTLA-4+ extracellular vesicles

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Dendritic cells (DCs) are critical mediators of immunity which bridge the gap between the innate and adaptive arms of the immune response. They not only participate in frontline (innate) defenses against pathogens and tumors, they also drive subsequent adaptive immune responses and dictate the strength and timbre of such responses. The manner in which dendritic cells tailor the specificity of adaptive immune responses is an area of intense investigation, as these mechanisms involve a conglomeration of external and internal signals that drive DC differentiation and maturation pathways, specifically with regard to the surface receptors and soluble inflammatory mediators expressed by DCs. Additionally, DCs release extracellular vesicles (EVs) which themselves are known to significantly influence the immune response in a variety of ways.
In our attempts to harness the therapeutic potential of these EVs, we have determined that the immunostimulatory effect of DC-derived EVs was downregulated by the presence of the immunosuppressive molecule, CTLA-4 on their surface. We have previously shown that the release of CTLA-4+ EVs is heightened under culture conditions that polarize monocyte-derived DCs in a Th2 manner, whereas the release of such EVs was significantly decreased under Th1-polarizing conditions. Our investigations determined that DC CTLA-4 is only expressed intracellularly and on the surface of DC-derived EVs in the extracellular space. In a B16 mouse melanoma model, antitumor immunity and survival were significantly heightened among recipients of a CTLA-4 siRNA-treated DC vaccine, relative to NT (non-targeting)-siRNA-treated DC vaccine recipients. Here, we show that DC-derived EVs alone can mediate T cell activation if devoid of CTLA-4. Specifically, we demonstrate that T cells are capable of taking up mature DC-derived EVs and that CTLA-4+ EVs, unlike their CTLA-4neg counterparts can suppress the expression of IFNÎ³ and Granzyme B by CD8+ T cells in vitro. Additionally, the loss of CTLA-4 in CD11c-expressing cells in mice results in early lethality in addition to heightened peripheral T-cell activation, aberrant tissue morphology, loss of cellularity in primary lymphoid organs, and the absence of pancreatic tissue at the time of death. These lines of evidence suggest that DC CTLA-4 and in effect, DC-derived EVs are key mediators of Th1 responses in vitro and in vivo.

Poster #38

Breaking the serotype barrier: PCPcon antigens stimulate broad spectrum protection against alphaviruses.

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Background: The mosquito-borne alphaviruses (AV) cause epizootic encephalitic outbreaks with high mortality (eastern and Venezuelan equine encephalitis (EEEV and VEEV), Madariaga virus (MADV)) or chronic arthralgia and heart problems (Chikungunya virus, CHIKV). As many AV co-circulate, there is a pressing need for safe, broad spectrum vaccines to prevent debilitating encephalitis and arthralgia. Human trials have shown immune interference when mixing antigens. We produced PhysicoChemical Property Consensus (PCPcon) protein and peptide antigens to epitope-rich regions of the E2 protein of all AV1and showed they generated serotype specific or broad-spectrum, neutralizing antibodies in rabbits as determined by plaque reduction neutralization assays (PRNT50/80) against 3 AV serotypes 2. Protein/peptide antigens are molecularly defined and easily stored requiring no animal cell culture3.
Purpose: Demonstrate PCPcon protein/peptide protect against challenge with encephalitic AV.
Objective: Test computationally derived PCPcon E2-B domain antigens combined with peptides to surface exposed areas of the E2-A domain for protection against VEEV and EEEV challenge.
Methods: Rabbits were inoculated 1x with 200 ug each protein/10 ug each of 4 A-region peptides in specially formulated FCA, and boosted 3x with 100 Î¼g protein/10 Î¼g each peptide in FIA. CD1 mice were inoculated with 3 x 20Î¼g doses in ASO3 adjuvant, 3 weeks apart, of species-specific proteins antigens VEEVcon, EEEVcon, CHIKVcon, a PCPcon based on all three of these (EVCcon) or Mosaiccon, AllAVcon of 24 AV reference strains altered to contain known epitopes of VEEV and CHIKV. A mixture of 5 Î¼g each of 4 peptides representing surface-exposed regions of the E2-A domain was administered 2x at weeks 4 and 7 to each protein-vaccinated animal. Negative controls were adjuvant only, positive were IRES-attenuated whole AV expressing the E proteins of VEEV or EEEV4. Mice were challenged with wild-type strains VEEVZPC738 or EEEV93939 three weeks after the third inoculation.
Preliminary Results: All inoculated with EEEVcon+peptides survived EEEV challenge with no weight loss or viremia 2 days after challenge. For those vaccinated with the recombinant protein antigens plus peptides, 4 of 5 CHIKVcon,3 of 5 VEEVcon, 3 of 5 Mosaiccon and 2/5 EVCcon mice survived. All proteins reduced viremia 2 days after challenge. All the protein/peptide inocula reduced viremia 2 days after VEEV challenge, but only 2/5 VEEVcon and 1/5 EEEVcon inoculated survived.
Conclusions: All the protein/peptide inocula induced serotype specific or broad-spectrum protective antibodies in inoculated rabbits. They also reduced viremia 2 days post challenge in inoculated CD1 mice but had mixed effects on survival. Further vaccination studies will use different adjuvants and optimized protein/peptides to enhance broad spectrum protection.

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Poster #39

scRNAseq reveals differences in gene expression of thymocytes between sexes, among inbred strains of mice and in mice exposed to an xenobiotic estrogen in utero

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Single cell RNA sequencing has revealed many properties of thymocytes and thymic development in mice but very few studies have compared thymic function among different strains of mice or between male and female mice. We designed an efficient study to investigate the thymic profiles of mice of both sexes from five conventional inbred strains with different predispositions to autoimmune diseases. We subsequently tested whether prenatal exposure to the endocrine disruptor diethyl stilbestrol (DES) altered the thymic profiles of either sex of the same strains of mice. Thymi were collected from 38 do age-matched male and female mice of inbred strains C57Bl/6J, MRL/MpJ, NOD/ShiLtJ; NZB/BINJ and SJL/J. Tissue samples were pooled by sex and immediately processed to liberate thymocytes and other thymic cells. Samples were assessed for cell viability and immediately used for library construction for scRNAseq using the 10X platform. Data were annotated using a custom pipeline based on strain-specific coding variants to assign each cell to strain of origin. Cells were categorized into 35 types by expression profiles using the ImmGen expression database. We noted numerous differences among strains, with notably fewer T cells in NZB, a 5-10-fold increase in double-positive T cells in male NOD, and an exponential increase in memory CD8 cells in MRL. The scRNAseq experiment was repeated with mice exposed in utero to 0.25 ug DES in corn oil at 11.5 days post-conception. The most notable difference between DES and control females was a 10-fold increase in double positive T cells in the treatment group across strains and greater than 20-fold increase in immature single-positive and immature gamma-delta T cells in the BL6 strain. (This work was funded, in part, by a grant from the National Institute of Environmental Health Sciences P30 ES029067).

Poster #40

A clinical presentation of idiopathic CD4 lymphocytopenia

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An otherwise healthy male (60s) presented five years prior to our encounter to the emergency department with stroke-like symptoms. Underlying cryptococcal meningitis infection was ultimately found leading to extensive work-up to rule out underlying malignancy and HIV infection. Results returned negative with the exception of a CD4 count less than 25. Several years later, he presented to the emergency department with fatigue. He was then found to have severe anemia with underlying mycobacterium avium complex (MAC) infection involving the bone marrow and a left psoas abscess. After multiple courses of antibiotic therapy targeted towards MAC, the infection persisted due to bone marrow involvement. By diagnosis of exclusion, he was eventually found to have idiopathic CD4 lymphocytopenia (ICL). Here we describe this condition, which has the potential to cause significant morbidity, and obligates the need for high clinical suspicion for timely diagnosis to enhance life quality and outcomes for patients.

Poster #41

Ablation of long noncoding RNA MALAT1 activates antioxidant pathway and alleviates sepsis in mice

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The metastasis-associated lung adenocarcinoma transcript1 (MALAT1) is a long noncoding RNA (lncRNA) and is known for its role in cancer development and prognosis. In this study, we report that MALAT1 plays an important role in regulating acute inflammatory responses in sepsis. In patient samples, MALAT1 expression was positively correlated with severity of sepsis. In cultured macrophages, LPS treatment significantly induced MALAT1 expression, while genetic ablation of MALAT1 greatly reduced proinflammatory cytokine levels. Furthermore, MALAT1-ablated mice had significantly increased survival rates in cecal ligation and puncture (CLP)-induced sepsis and LPS-induced endotoxemia. One novel and salient feature of MALAT1-ablated mice is greatly reduced ROS level in macrophages and other cell types and increased glutathione/oxidized glutathione (GSH/GSSG) ratio in macrophages, suggesting an increased antioxidant capacity. We showed a mechanism for MALAT1 ablation leading to enhanced antioxidant capacity is through activation of methionine cycle by epitranscriptomical regulation of methionine adenosyltransferase 2A (MAT2A). MAT2A 3â€²UTR can be methylated by METTL16 which was known to directly bind to MALAT1. MALAT1 ablation was found to reduce methylation in MAT2A hairpin1 and increase MAT2A protein levels. Our results suggest a MALAT1-METTL16-MAT2A interactive axis which may be targeted for treatments of sepsis.

Poster #42

Characterizing spatial and temporal host innate immune responses to SARS-CoV-1 and -2 infection in pathologically relevant human lung epithelial cells

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Severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1) and -2 are beta-coronaviruses (Î²-CoVs) that have caused significant morbidity and mortality worldwide. Therefore, a better understanding of host responses to Î²-CoVs would give insights into the pathogenesis of these viruses and identify potential targets to develop medical countermeasures. Our objective was to use a systems biology approach to explore the magnitude and scope of innate immune responses triggered upon SARS-CoV-1 and -2 infection over time in pathologically relevant human lung epithelial cells (Calu-3/2B4 cells) through RNASeq and bioinformatics applications.

RNA from virally infected-Calu-3/2B4 cells was analyzed using the Illumina NextSeq550 platform with polyA+ mRNA sequencing libraries on a single 400 million read. Genes with an expression value of more or less than a log2fold change of Â± 1.5 and statistically significant p-value of < 0.05 were selected for QIAGENâ€™s Ingenuity Pathway Analysis (IPA). After performing IPA, we chose differentially expressed genes (DEGs) related to infectious diseases and immunity and performed functional enrichment analysis through Cytoscape App ClueGO.

The results of our study demonstrate that SARS-CoV-1 and -2 stimulates similar yet distinct innate antiviral signaling pathways in pathologically relevant human lung epithelial cells. Upregulated genes found at multiple time points were related to the regulation of the viral life cycle, interferons, and interferon-stimulated genes (ISGs), and we identified four ISGs of interest. Moving forward, these ISGs will be investigated as potential targets against Î²-CoVs.

Poster #43

Coxiella burnetii virulent phase I and avirulent phase II bacteria differentially interaction with neutrophils

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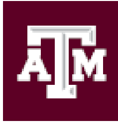
Coxiella burnetii is an obligate intracellular Gram-negative bacterium that causes a worldwide zoonosis, Q fever. Although human Q fever can develop into a severe, chronic and potentially fatal disease, there is currently no vaccine commercially available for the prevention of Q fever in the US. Additionally, it is difficult to treat chronic Q fever patients with various antibiotic regimens. Therefore, there is an urgent need to develop alternative prophylactic and therapeutic strategies for the prevention and treatment of human Q fever. Neutrophils are considered to be the major effector cells of the innate immune system, with a primary role in resistance to extracellular bacterial pathogens. However, despite the obligate intracellular lifestyle of C. burnetii, our previous study demonstrated that neutrophils play a critical role in host defense against C. burnetii infection. C. burnetii Nine Mile strain undergoes a lipopolysaccharides (LPS) phase variation in which its virulent smooth LPS phase I (NMI) strain converts to an avirulent rough LPS phase II (NMII) strain upon serial passage in vitro culture systems. To understand the mechanisms of C. burnetii interaction with neutrophils, we examined if NMI and NMII bacteria differentially interact with neutrophils. Our results demonstrated that i) neutrophil accumulation correlates with the ability of virulent NMI and avirulent NMII bacteria to induce an inflammatory response and cause disease in mice; ii) NMI-infected neutrophils survive longer than NMII-infected neutrophils in vitro; iii) the genes involved in autophagy related pathways are upregulated in NMII-infected neutrophils but downregulated in NMI-infected neutrophils; iv) NMII infection may activate autophagosome maturation via LC3 lipidation while NMI infection does not; and v) both NMI and NMII strains can inhibit or delay neutrophil programming death but the NMI strain showed a stronger ability than the NMII strain. These findings suggest that modulating autophagy and apoptosis in neutrophils may be an important pathogenic factor for virulent C. burnetii to establish a persistent infection and cause clinical disease in animals.

ACKNOWLEDGEMENTS

Garry Adams
Yeon Choi
Mike Criscitiello
Paul deFigueiredo
Stacy De Leon
Jessica Galloway-Pena
Peter Hotez
Stefan Kaufman
LeeAnn Nichols
Kristin Patrick
Jim Samuel
Ashley Seabury
Sarguru Subash
Christina Sweeney
Phillip West



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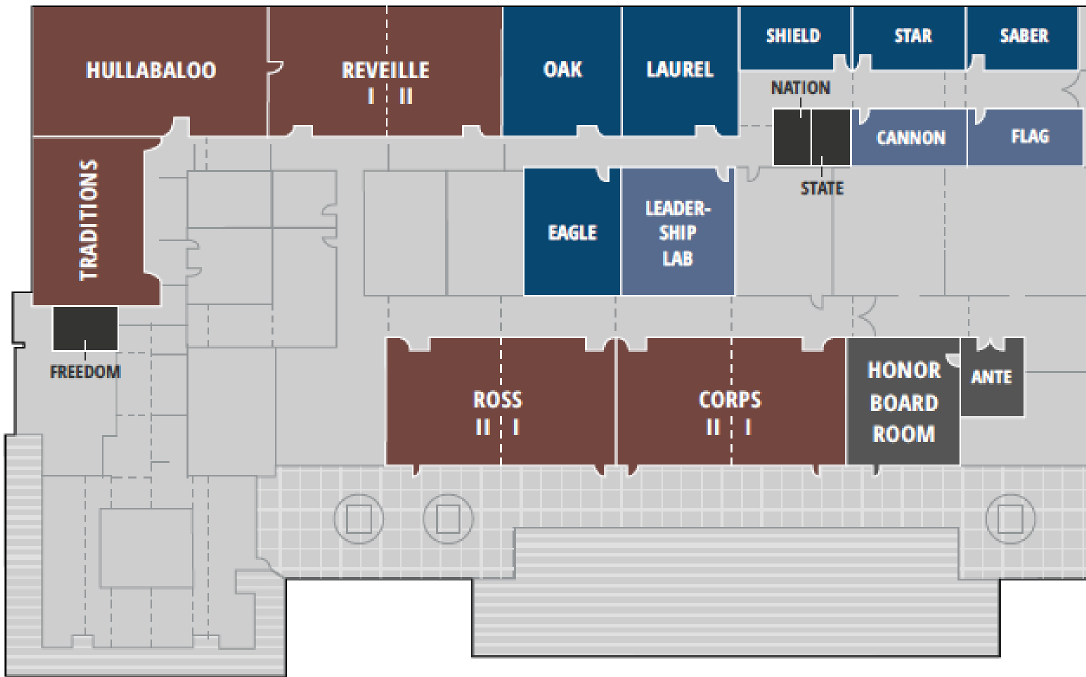

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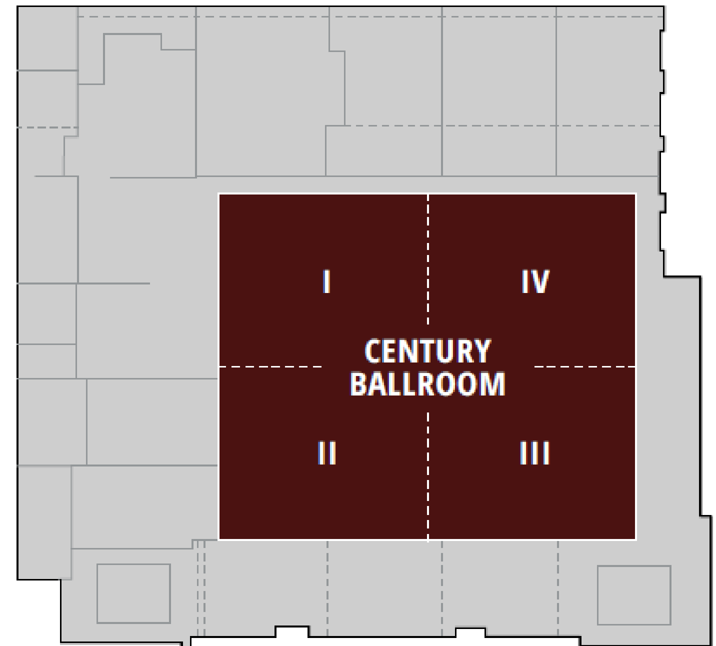

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