### Immune Responses that Protect against Sexually Transmitted Chlamydia Infection: Implications for Vaccine Design

Richard P. Morrison, Sandra G. Morrison, and Elizabeth K. Naglak

Chlamydia trachomatis is the most commonly reported sexually transmitted bacterial infection. Despite significant advances in our understanding of the biology and antigenic structure of chlamydia, and the epidemiology and clinical spectrum of disease, the magnitude of the morbidity from *C. trachomatis* infections remains an important public health concern. It is estimated that greater than 100 million new chlamydia infections occur annually worldwide, 2.8 million new infections annually in the US alone. Women are particularly at risk because of their propensity to develop post-infection complications such as pelvic inflammatory disease, ectopic pregnancy and infertility. The cost associated with treating genital chlamydia infection and the associated complications exceeds $500 million annually in the US. Although chlamydia infection is readily cured with antibiotics, control measures based upon antimicrobial chemotherapy alone are hampered by the high frequency of asymptomatic infections and delayed diagnosis. However, definitive control of chlamydia sexually transmitted infection is possible through the development of a safe and effective vaccine. Toward this goal, the Morrison laboratory is: i) defining immune responses that confer protective immunity to experimental chlamydia infection; ii) analyzing Chlamydia mutants to identify key virulence factors; and iii) assessing the human immune response to identify correlates of protective immunity.

Showcase of Medical Discoveries:
Infectious Disease & the Microbiome
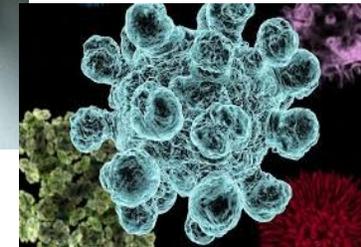
Wednesday, September 16, 2015
4:30—6:00 p.m.

A Wine & Cheese Reception Featuring UAMS Investigators Discussing their Research and Discoveries.

Winthrop P. Rockefeller Cancer Institute
10th Floor Rotunda   4:00-5:30 p.m.

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Does the Gut Microbiome Mediate Associations of Menarche with Cardiovascular Disease Risk in Postmenopausal Women?

Yan Wang, Robert Delongchamp, Mohammed El Faramawi, Mohammed Orloff, Jordana Bell, and Barbara Fuhrman

Early menarche is a known risk factor for cardiovascular disease (CVD). During childhood, the evolving gut microbiome may affect growth and development through its impact on energy balance and metabolism of endogenous hormones. In adulthood, it plays known roles in the development of CVD, as a modulator of the impact of diet on atherosclerosis and a cause of systemic inflammation. We hypothesize that the association of early menarche with CVD may reflect a shared cause, which is reduced gut microbial diversity. We have proposed to test this hypothesis in existing data from the Twins UK study, which includes 2,747 adult female twins. They provided fecal samples for 454-pyrosequencing of microbial 16S rRNA, reported their age at menarche, and underwent measures of cardiovascular risk including blood pressure, lipids, waist circumference, fasting glucose and insulin. Microbial parameters include measures of alpha diversity, beta diversity, and metabolic activity of the gut microbiome. We use linear regression modeling to test whether microbiome measures are associated with menarche and then use mediation analysis to formally test whether the microbial measures mediate observed associations of menarche with cardiovascular risk markers.

**_The Living Lung: A Novel Approach to Studying Coxiella burnetii Interactions with Humans_**

Daniel E. Voth, Joseph G. Graham, Caylin G. Winchell, Uma M. Sharma, Richard C. Kurten, and Joseph G. Graham

Our laboratory studies the vastly understudied bacterial pathogen _Coxiella burnetii_ that causes human Q fever, a flu-like illness that can progress to chronic heart disease. _Coxiella_ is a highly infectious category B select agent and no vaccine is approved for prevention of Q fever in the United States. _Coxiella_ grows inside human lung cells and without these cells cannot cause disease. Thus, identification of novel events within infected cells will provide new treatment targets. We are pioneering characterization of the human immune response to _Coxiella_ using two new infection platforms developed in our laboratory: functional human lung tissue slices and human alveolar macrophages. We are the only _Coxiella_ laboratory using these disease relevant systems and this research has been met with great enthusiasm. Using these approaches, we are identifying numerous small compounds that prevent _Coxiella_ growth in macrophages. Preventing growth is the critical step to preventing disease, linking our research to eventual medical application. We are also studying the role of secreted _Coxiella_ proteins in disease. These secreted proteins represent new potential targets for disease intervention. These areas, and our newly developed infection platforms, are opening new doors into the interplay between intracellular pathogens and humans.

Defining the Balance Between Health and Disease in Infection-Associated Cancers
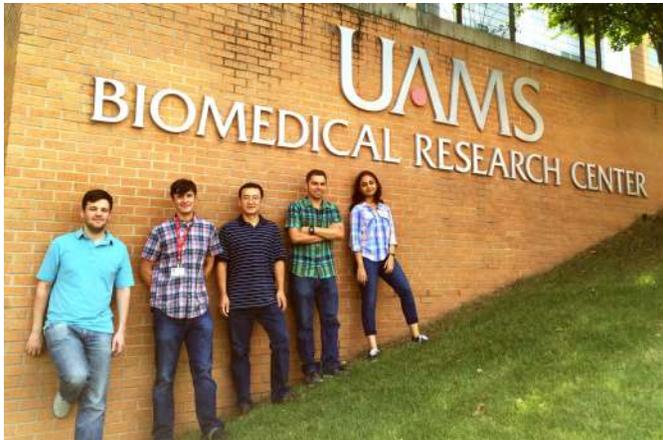
Jeffrey Sifford, Eduardo Salinas, Gang Li, Arundhati Gupta, Drew Stahl, Shweta Chavan, Rick Edmondson, and J. Craig Forrest

Essentially all adult humans are chronically infected with viruses that are known to cause cancer. This includes Epstein-Barr virus and Kaposi sarcoma-associated herpesvirus, gammaherpesviruses that establish life-long infection of cells in the immune system and thereby place the host at risk for numerous cancers and lymphoproliferative diseases. Research in the Forrest laboratory seeks to understand key factors that regulate the delicate balance between host control of chronic gammaherpesvirus infection and virus-driven disease, and we are doing so in three main focus areas. First, we are defining the roles played by host tumor suppressor proteins, such as p53, in limiting chronic infection, virus-driven cellular proliferation and oncogenesis. Second, as a converse and complementary approach, we are determining how specific viral proteins thwart host defense mechanisms to allow life-long infection. Third, we are developing and applying state-of-the-art technologies as a means to enhance a systems-level comprehension of the cellular and viral processes that regulate infection and disease. Through our studies we will gain a new and improved understanding of both viral and host-regulated steps that dictate the outcomes of long-term infection by gammaherpesviruses, thereby providing more informed or novel therapeutic options to treat virus-triggered cancers.

Effects of Obesity on Gut Microbiota using the Obese Zucker Rat Model

Reza Hakkak[1,2,3], Soheila Korourian[4], Steven Foley[5], and Bruce Erickson[5]

[1]Dept. of Dietetics and Nutrition, [2]Arkansas Children Hospital Research Institute, [3]Dept. of Pediatrics, [4]Dept. of Pathology, UAMS, and [5]National Center for Toxicological Research, U.S. FDA, Jefferson, AR

Obesity has been epidemic in the United States for more than two decades. Previously, we reported that obesity promotes DMBA-induced mammary tumor development using the obese Zucker rat model. The intestinal microbiota is composed of a diverse population of obligate and facultative anaerobic microorganisms, and these organisms carry out a broad range of metabolic activities. Obesity has been linked to changes in the intestinal microbiota, but the composition of the bacterial population in lean and obese Zucker rats has not been carefully studied. Therefore, the study objective was to determine the effects of obesity on the gut microbiota in this model strain. Lean and obese female Zucker rats (n=16) were assigned to AIN-93-G diet for 8 weeks. Fecal samples were collected at the beginning and end of the experiment. Quantitative group-specific real-time PCR and 16S rRNA sequencing were used to evaluate the composition of the fecal bacterial populations. Differences in the Bacteroidetes/Firmicutes ratios and levels of Actinobacteria present were associated with the subsequent lean or obese state. Our preliminary results suggest that there are differences between gut microbiota of the lean and obese rats using the Zucker rat model. Further investigation will be needed to determine the effects of obesity on gut microbiota in relation to DMBA-induced mammary tumor formation.

Dysbiosis Induced Impairment of Immune Surveillance in Non-gastrointestinal Tumors

Samir Jenkins, Carl Cerniglia, Kieng B. Vang-Dings,
Robert J. Griffin, and Ruud P.M. Dings

Gut microbiota profoundly shapes immunity in humans. Dysbiosis, a disturbance in the quantity and composition of gut microbiota, leads to aberrant immune development and functioning and subsequently increases the risk of various diseases, including cancer.

A growing body of evidence has started to indicate the involvement of dysbiosis in colon carcinogenesis and progression by favoring chronic inflammation and immune suppression. However, the effects of dysbiosis on non-gastrointestinal (non-GI) cancers are largely unknown. Moreover the mechanistic effects of dysbiosis on stromal immune surveillance in non-GI primary tumor micro-environment, is unexplored territory. Since diminished immune surveillance, i.e. leukocyte extravasation, results in enhanced tumor progression, overcoming this dysbiosis-induced impairment will enhance tumor immunity and has potential to improve cancer treatment efficacy and subsequently patient survival and quality of life.

## Association of miRNA Expression P)rofile with Chlamydial-induced Genital Tract Pathology

Laxmi Yeruva, Anthony Maurelli, Nicole Spencer, Nancy Praskievicz, Grant McChesney, Garry, Meyers, and Roger G. Rank

Currently, with respect to chlamydial infections, one cannot predict the probability of an infected patient developing pelvic inflammatory disease. To determine if specific biomarkers may be associated with distinct chlamydial pathotypes, we utilized two Chlamydia muridarum variants (M4, M9) that showed differences in the development of upper tract pathology. M4 has a slower growth rate in vitro, induces pathology in 6 of 30 C57Bl/6 oviducts versus 25 of 30 oviducts in M9-infected mice. To determine if chemokine cytokine production within 24 hr of infection will define the outcome of pathology, 15 chemokines and cytokines were measured. M4 infection induced significantly lower levels of CXCL 1, CXCL 2, TNF-a and CCL 2 in comparison to M9 infection with similar ribosomal RNA (rs16) levels for chlamydiae. To understand if chemokine cytokine responses would also be reflected in miRNA expression profile within 24 h of infection, 134 inflammation-related miRNAs were measured. Interestingly, 12 miRNAs (miR-135a-5p, miR155-5p, miR325-3p, miR338-5p, miR105, miR132-3p, miR142-3p, miR142-5p, miR147-3p, miR223-3p, miR298-5p and miR299a-3p) were expressed significantly higher during M4 infection than M9 infection, inversely correlating with the respective chemokine/cytokine responses. To our knowledge this is the first report demonstrating that early biomarkers elicited in the host could differentiate between two pathologic variants of chlamydiae and be predictive of upper tract disease.

Use of a Novel Bone-Targeting Agent for the Enhanced Delivery of Vancomycin to Bone
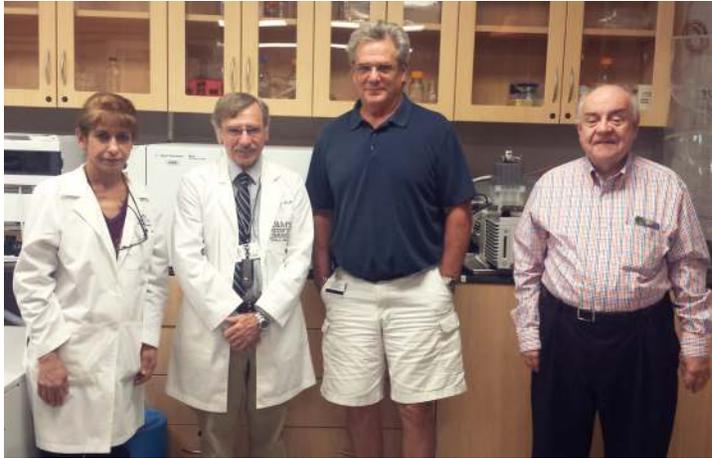
Zaineb A. F. Albayati, Manjula Sunkara, Suzannah M. Schmidt-Malan, Melissa J. Karau, Andrew J. Morris, James M. Steckelberg, Robin Patel, Philip J. Breen, Mark S. Smeltzer, K. Grant Taylor, Kevyn E. Merten, William M. Pierce, and Peter A. Crooks

The objective of the present study was to determine the extent to which a novel bone targeting agent could be used to enhance the accumulation of vancomycin in bone after systemic administration. Albino Wistar male rats were given a single IV injection of 50 mg/kg vancomycin or the molar equivalent of BT-2-minipeg-2-vancomycin (BT-vancomycin, Fig. 1). Plasma and the left tibia were collected 1, 6, 12, 24, 72 and 168 h after injection. Pharmacokinetic (PK) analysis estimated that vancomycin had a maximum concentration (C_{max}) of 12.63 ± 2.38 μM at a peak time (T_{max}) of 1 h, a clearance (Cl_{tot}) of 0.65 ± 0.12 L/h/kg, a half-life ($t_{1/2}$) of 1.44 ± 0.09 h and an area under the curve (AUC) of 58.71 ± 10.33 h·μM. In contrast, PK analysis after administration of BT-vancomycin confirmed a reduction in Cl (0.048 ± 0.01 L/h/kg), a longer $t_{1/2}$ of 21.14 ± 4.86 h, and an increased AUC of 631.39 ± 95.13 h·μM. Additional studies afforded similar PK profiles after IP injection using a multiple dosing regimen (seven doses at 12 h intervals). These results demonstrate that BT-vancomycin is a potential carrier for the systemic delivery of vancomycin in the treatment of bone infections.

The Center for Microbial Pathogenesis and Host Inflammatory Responses
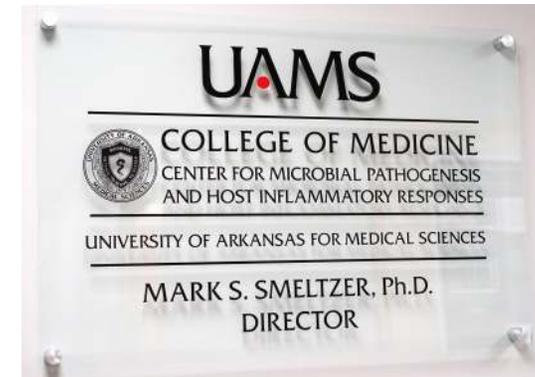
Mark S. Smeltzer, Ph.D. and Richard P. Morrison, Ph.D.

The UAMS Center for Microbial Pathogenesis and Host Inflammatory Responses (CMPHIR) was established in August 2015 with a five-year grant of over $10,000,000 from the National Institute of General Medical Sciences (NIGMS). CMPHIR's goal is to address the increasing threat to human health of infectious diseases. There is an urgent need for novel methods to combat infectious diseases, and our underlying scientific hypothesis to address this need requires a complete understanding of the microbial pathogens that cause infections and the manner in which they interact with the host causing adverse immunological and inflammatory consequences. In its first three years, CMPHIR has supported 12 investigators at UAMS and Arkansas Children's Hospital Research Institute (ACHRI) that study host response to bacterial, viral and protozoan infections. To facilitate these studies, the Center has significantly expanded research capabilities by purchasing instrumentation to upgrade key Core facilities (flow cytometry, genomic sequencing, proteomics), and establishing new shared resources (microscopy).

The Center's impact on infectious disease research on the UAMS campus has been manifold and evidenced by the large number of Showcase participants that have received CMPHIR research funding and by those investigators' success in obtaining additional NIH and other extramural grants; CMPHIR investigators have obtained $6,714,420 in extramural funding in the last three years. Moreover, an additional $6,679,639 in extramural funding has been approved and currently under final consideration by the granting agencies. Thus, in only 3 years, CMPHIR has brought an additional $13+ million in research dollars to UAMS. The CMPHIR has had, and will continue to have, a remarkable scientific and economic impact on UAMS, ACHRI, and the state of Arkansas.

### Defeating Staphylococcus aureus as an Orthopaedic Pathogen

Mark S. Smeltzer, Karen E. Beenken, Allister Loughran,

Daniel Meeker, and Danielle Atwood

The goal of the Smeltzer laboratory is to understand the pathophysiology of *Staphylococcus aureus* orthopaedic infections to a degree than can be exploited to therapeutic advantage. We have focused much of our work on understanding the mechanisms by which *S. aureus* forms a biofilm, a key component of bone and implant-associated infections. This has led to three primary areas of research. The first is the identification of small molecule inhibitors of *S. aureus* biofilm formation, which is a key factor contributing to the unique problem of orthopaedic infections. The second is to define *how* S. aureus forms a biofilm in the body, the logic being that specific factors required for this process may also represent viable therapeutic targets. The third is based on development of an antibody-based nanotherapeutic strategy that targets biofilm-associated surface proteins to achieve targeted localization of antibiotic-loaded gold nanocages directly to bacterial cells. This allows us to generate laser-assisted photothermal effects and the simultaneous synergistic release of antibiotics while at the same time avoiding systemic toxicity. Research in the Smeltzer laboratory is currently funded by the National Institute of Allergy and Infectious and the Department of Defense through the Congressionally Directed Medical Research Program.

Myxoma Virus Differentially Influences Human CD14+ Myeloid Cells from Healthy Donors and Ovarian Cancer Patients
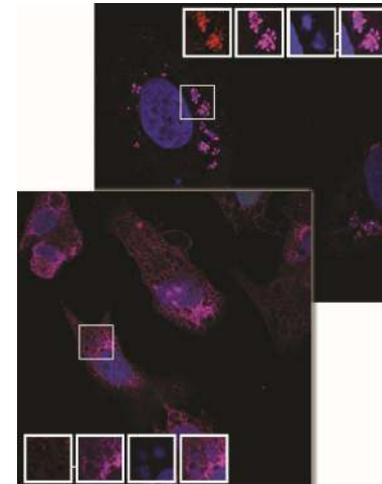
Shana Chancellor, Jason Liem, Bernice Nounamo, Martin Cannon, and Jia Liu

Despite decades of treatment development, ovarian cancer (OC) remains the most deadly gynecological malignancy among women in the US. The difficulty in OC treatment is largely due to the heterogeneity of the disease, but a common feature found among OC patients is the immunosuppressive tumor microenvironment. OC-associated CD14+ myeloid cells play an important role in disease progression by regulating the immunosuppressive tumor environment. Myxoma virus (MYXV), a rabbit specific poxvirus, is a candidate of oncolytic virus for cancer treatment. We examine the immunotherapeutic potential of wildtype MYXV and two engineered MYXVs in influencing OC-associated CD14+ cells in this study. Healthy donor CD14+ and OC patient ascites-associated CD14+ cells were mock treated, or infected with either wildtype or an engineered MYXV. We then examined the response of these CD14+ cells to infection on cytokine secretion and gene expression profile. We found that healthy and disease CD14+ monocytes are very different in cytokine secretion profile with or without MYXV infection. Importantly, infection by MYXVs significantly attenuated disease CD14+ cells from secreting cytokines that regulate the tumor environment. This observation suggests a potential application of MYXV immunotherapy targeting OC-associated monocytes. We are currently testing MYXV in pre-clinical models of OC metastasis.

Poster #11

Rhinovirus Infection Induces Th2-Promoting Innate Cytokines in Ex Vivo Precision Cut Lung Slice Airways

JL Kennedy, E Brown, M Kurten, SM Jones, RC Kurten

Introduction: Rhinovirus (RV) is associated with asthma exacerbations, but little is known about the cellular response to virus leading to exacerbations. We hypothesized RV39 infection of airways in precision cut lung slices (PCLS) from asthma subjects would induce a pro-allergic cytokine signature.

Study Design/Methods: PCLS from donors with and without reported asthma were prepared from human lungs. Explants were infected with RV39, and viral loads measured using qPCR. mRNA levels for IFN-l, IL-15, IL-33, IL-25, TSLP, and IL-13 were measured and normalized to uninfected airways from the same subject and b-actin. Results are expressed as means and standard deviations of these values (DDCT).

Results: qPCR for RV39 confirmed active infection of PCLS at 24 hours (53,330+70,491 virions/mL cDNA). At 24 hours, mRNA expression of IL-15, IL-33, and IFN-l were similarly induced in asthmatics and controls. IL-25 (asthma 3.8+3.78; control -2.4+5.08), TSLP (asthma 4.2 +3.29; control -2.1+3.69), and IL-13 (asthma 3.8+4.16; control -3.7+4.06) were induced only in PCLS from asthmatics.

Conclusions: In PCLS airways from subjects with reported asthma, RV39 infection enhanced mRNA expression of IL-25, TSLP, and IL-13. We suggest that IL-25 and TSLP increase IL-13 expression by mast cells, innate lymphoid type 2 cells, and/or T cells in PCLS airways of asthma donors.

Poster #6

Identification of Host Membrane-trafficking Genes Involved in Coronavirus Infection
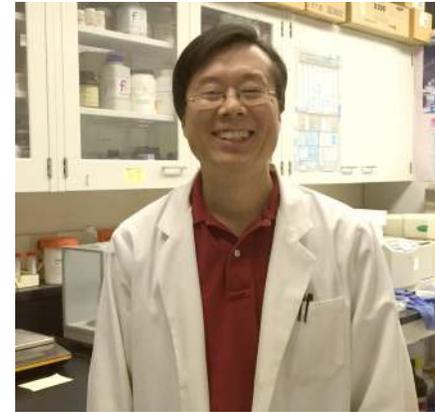
Jianzhong Cao, Ph.D and Xuming Zhang, Ph.D

Historically, Coronavirus mouse hepatitis virus serves as an animal model for studying neurodegenerative diseases such as multiple sclerosis. It also serves as a safe surrogate for developing antiviral drugs to the highly pathogenic human coronaviruses that cause Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS). As the membrane-trafficking system is known to participate in coronavirus life cycle (from entry to replication to spread), we employed a recombinant coronavirus and a custom-made siRNA library targeting 148 host membrane-trafficking genes for identifying antiviral drug candidates. We found that 60 genes are involved in viral entry. They have diverse cellular functions, ranging from actin polymerization and Golgi-trafficking to endocytosis. Additional 30 genes were identified that are involved in viral replication and/or entry. These include members of the Ras family, COG complex, and cargo sorting. The roles of some of the candidate genes in coronavirus life cycle were further established by using available chemical inhibitors. Findings from this study thus allows us to further elucidate the molecular mechanisms by which these host genes regulate coronavirus infection. It also facilitates the discovery of a new class of antiviral drugs for treatment of SARS and MERS.

### *Mechanisms of Reovirus Bloodstream Dissemination*

Karl W. Boehme, Joseph Koon, Matthew B. Phillips, and Johnasha D. Stuart

Viral spread from mucosal sites of infection to target tissues and organs is a fundamental step in viral pathogenesis. Viruses typically use the bloodstream and lymphatic system as the pathway for delivering virus to organs and tissues throughout the body. The overall objective of our work is to define the mechanisms and functional consequences of viral spread via the blood. Mammalian orthoreoviruses (reoviruses) provide a highly tractable model system for studies of viral replication and pathogenesis. Reoviruses spread systemically from the intestine or lung by a combination of hematogenous and neural routes to every organ system in the body, including the central nervous system (CNS). We identified reovirus nonstructural protein σ1s as a viral factor that is required for reovirus spread by hematogenous routes. We also found that σ1s promotes reovirus replication by enhancing viral resistance to type-1 interferon responses. Our work suggests that type-1 interferon responses represent a critical host barrier that reovirus must overcome to access the bloodstream and disseminate to target organs and tissues. This research will have broad general impact in uncovering unifying principles that govern systemic viral spread.

Tracking Antigen-specific CD4 T Cell Responses to Chlamydia Female Reproductive Tract Infection
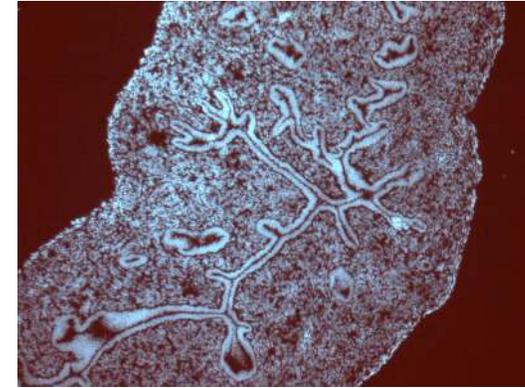
Lin-Xi Li, Ph.D., Dept. of Microbiology and Immunology, Center for Microbial Pathogenesis and Host Inflammatory Responses, UAMS

Stephen J. McSorley, Ph.D., Center for Comparative Medicine, School of Veterinary Medicine, University of California, Davis

Sexually transmitted Chlamydia infections are increasingly prevalent in the US and a major cause of infertility in young women. To date, no Chlamydia vaccine is available. Greater understanding of the adaptive immune response to Chlamydia female reproductive tract (FRT) infection will be required if an effective vaccine is to be developed. Here, we generated novel tools called Chlamydia-specific MHC class-II tetramers, which for the first time allow direct visualization of the endogenous, antigen-specific CD4 T cell response to *Chlamydia muridarum* FRT infection. Chlamydia-specific CD4 T cells expanded rapidly and persisted as a stable memory pool for months after systemic infection. The majority of expanded Chlamydia-specific CD4 T cells exhibited a Th1 phenotype and produced IFN-γ, TNFα and IL-2, While most lymph node Chlamydia-specific CD4 T cells expressed Th1-specific transcription factor T-bet, a small percentage co-expressed regulatory T cell marker Foxp3, and RORγt-expressing Th17 cells were enriched within the FRT. We are currently focusing our efforts on understanding memory CD4 T cell responses to Chlamydia FRT reinfection.

Poster #9

Memory follicular helper T cells promote rapid antibody production after secondary Plasmodium yoelii infection

Daniel J. Wikenheiser and <u>Jason S. Stumhofer</u>

Since 2004, protection from secondary blood-stage infection with *Plasmodium yoelii* is predominately antibody-mediated. Long-lived plasma cells generated during primary infection are responsible for maintaining parasite-specific antibody titers that are present upon secondary infection. However, the contribution of *de novo* antibody production during secondary infection is a poorly defined process. Follicular helper T (TFH) cells promote B cell survival, and facilitate somatic hypermutation and affinity maturation within germinal centers (GC) of secondary lymphoid organs, resulting in production of high-affinity antibody after infection. While the majority of the TFH cell population declines over time after resolution of a primary infection, a population of TFH cells with a memory phenotype emerges. However, the precise role of memory TFH cells during secondary humoral responses is not fully understood. Here, we demonstrate that adoptive transfer of central memory T cells (TCM) that express CXCR5, a marker of TFH cells, and memory B cells into naive wild-type mice promoted early plasma cell and GC B cell differentiation after P. yoelii challenge. Furthermore, a greater percentage of transferred CXCR5+ TCM cells up-regulated the transcription factor Bcl6, which is associated with TFH cell differentiation, compared to transferred CXCR5- TCM cells. Co-transfer of CXCR5+ or CXCR5- TCM cells with memory B cells into naive Cd28-/- mice resulted in production of higher amounts of isotype-switched antibodies that also had a higher affinity for antigen compared to mice receiving no cells or memory B cells only after *P. yoelii* challenge. Collectively, these data suggest memory TFH cells play an active role in secondary humoral responses by promoting rapid production of protective, isotype-switched antibodies with higher affinity for antigen.

Poster #8

Identifying Virulence Determinants in the Relapsing Fever Spirochete, Borrelia turicatae

Jacob I. Latham, and Danielle E. Fortune, PhD, Marissa Podgorski, and <u>Jon S. Blevins, PhD</u>

Pathogens of the genus *Borrelia* are spirochetal bacteria transmitted to humans via the bite of an infected arthropod. There are two infections, Lyme disease and relapsing fever, which are commonly associated with *Borrelia* infection. Lyme disease presents with numerous chronic manifestations, including rheumatologic, neurological, and cardiac abnormalities. Humans develop Lyme disease after becoming infected with *B. burgdorferi,* which is transmitted via the bite of *Ixodes sp.* hard ticks*.* In contrast, relapsing fever is an acute infection presenting as recurring episodes of fever, aches, and nausea with each episodes of fever being associated with bacteremia. In the United States, relapsing fever is caused by three species of *Borrelia* that are each transmitted by *Ornithodoros sp.* soft tick.

Despite intensive research efforts studying the *Borrelia* that cause Lyme disease and relapsing fever, there is still significant progress to be made towards under-standing pathogenic mechanisms that these bacteria utilize to cause disease, colonize tick vectors, and transmit during tick feeding. Specifically, we are working to determine how *Borrelia* controls the expression of its genes as it moves between tick and mammal and define contributions of individual *Borrelia* genes to bacterial virulence and tick colonization. One of the most direct approaches to demonstrate a causal relationship between a bacterial gene, its cognate gene product, and a requirement during the bacterial lifecycle is to create *Borrelia* strains in which specific genes of interest have been inactivated. The abilities of these mutant strains to colonize animals and ticks can then be assessed experi-mentally to determine whether a given mutant is no longer competent for infection. Once bacterial factors required for *Borrelia* infection or tick transmission have been identified, we can begin to study their physiological contributions to these bacterial processes. In addition, since these factors are known to be essential during the bacterial lifecycle, they also represent viable targets against which therapies could be developed to prevent or treat disease.