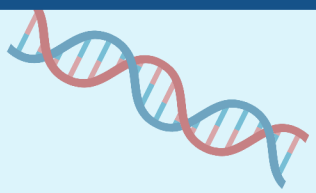
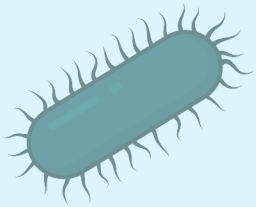
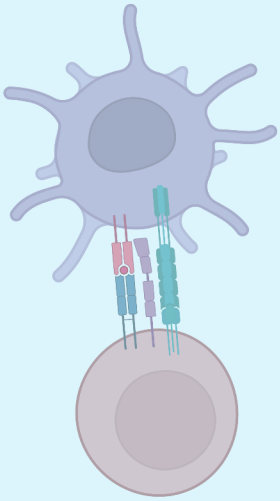
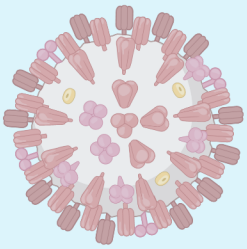
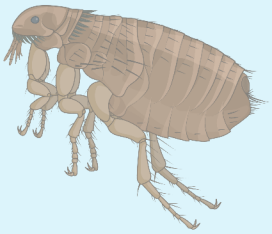
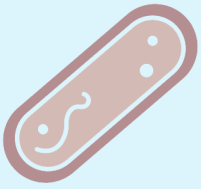
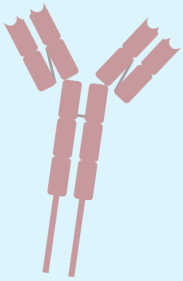




Microbiology and Immunology

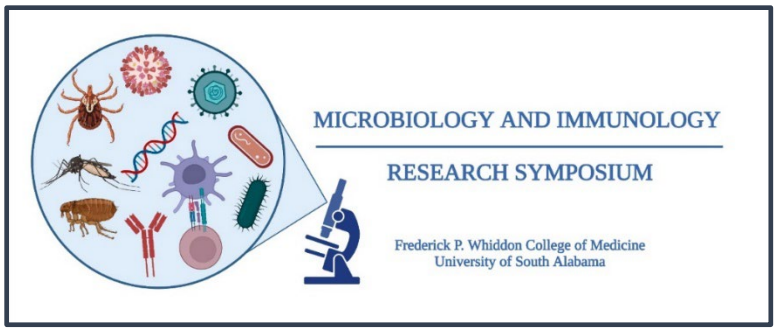
Research Symposium

Abstract Book

May 9, 2024



May 9, 2024

Student Center Terrace Room

2024 Infectious Disease and Host Defense (IDHD) Research Symposium

12:00 PM Dr. Allyson Shea, Microbiology and Immunology Assistant Professor and IDHD Committee Chairwoman, gives opening remarks and introduces the symposium keynote speaker.

12:15 PM Keynote Presentation "Rediscovering rickettsiae" by Dr. Christopher Paddock, Lead of the Diagnostic and Microbiology Team, Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention (CDC).

1:00 PM Intermission.

1:15 PM Student Research Presentations begin.
First Presentation—Rachel Rodenberg
Second Presentation— Nam Suwanbongkot
Third Presentation— Meagan Taylor
Fourth Presentation— Killian Brewer
Fifth Presentation— Shovon Lal Sarkar

2:30 PM Intermission.

2:40 PM Student Research Presentations resume.
Sixth Presentation—Amanda Tuckey
Seventh Presentation—Steven Smith
Eighth Presentation—Parker Norman
Ninth Presentation—Bailey Hettinger

3:40 PM The 2024 IDHD Symposium concludes. Dr. Shea gives closing remarks, and attendees transition to the Faculty Club for the Award Ceremony.

4:00 PM 2024 IDHD Research Symposium Award Ceremony begins.

6:00 PM 2024 IDHD Research Symposium Award Ceremony concludes.

γδ T17 cells are master regulators of the acute antiviral response in HSV-1 infected corneas

Rachel R. Rodenberg[1], Domenico Spadafora[2], Steffani Fitzpatrick[1], Grant Daly[3], Robert Lausch[1], Robert A. Barrington[1, 2]

[1]Department of Microbiology and Immunology, Frederick P. Whiddon College of Medicine, University of South Alabama Mobile, AL 36688
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Herpes stromal keratitis (HSK), caused by herpes simplex virus 1 (HSV-1) infecting the cornea, is the leading cause of infectious blindness in developed countries. Innate-acting γδ T17 cells are critical for protection against ocular HSV-1 infection. It is well established these cells can facilitate neutrophil influx into the cornea, but if/whether γδ T17 cells regulate other innate immune cells following ocular HSV-1 infection remains unclear. Natural killer (NK) cells also participate in the innate immune response generated against HSV-1 by directly killing infected cells by secretion of granzymes and promoting the antiviral response by production of IFN-γ. Our data strongly suggest that γδ T17 cell secretion of IL-17A promotes NK cell accumulation in the infected cornea. This is demonstrated in several ways: 1) in mice that lack γδ T cells (TCRδ$^{-/-}$) there are fewer antiviral NK cells following corneal HSV-1 infection compared to wild-type (WT) mice; 2) administering IL-17A to TCRδ$^{-/-}$ mice restored the NK cell population; and 3) neutralization of IL-17A in WT mice diminished NK cell accumulation leading to increased viral titers. Lastly, we observed that the absence of IFN-γ was associated with enhanced innate IL-17A production. In sum, this study identifies a novel mechanism by which γδ T17 cells regulate NK cell accumulation by secretion of IL-17 thereby limiting their own activity in the HSV-1 infected cornea.

Spotted fever group *Rickettsia* transmission and dissemination kinetics during infected tick feeding

Chanakan Suwanbongkot and Kevin Macaluso

Department of Microbiology and Immunology, Frederick P. Whiddon College of Medicine, University of South Alabama, Mobile, AL, United States

Rickettsia parkeri, an emerging bacterial pathogen, is transmitted by *Amblyomma maculatum* via infected tick saliva. Through an unknown route/mechanism, bacteria deposited in the host dermis during tick feeding disseminate via the bloodstream and infect the vascular endothelium, causing severe vasculitis in the major organs. Under laboratory conditions, *R. parkeri* transmission from infected ticks to vertebrate hosts occurred as early as 12-24 hours of feeding by the immature stage and 48 hours by adult ticks. Dissemination to the internal tissues was observed at 24 hours post-tick feeding. While the previous work was essential to outline transmission kinetics, the assays typically used multiple ticks. Thus, the minimal time for transmission of *R. parkeri* during the early phase of tick feeding (first 24 hours) and the actual amount of rickettsiae inoculated by an individual tick, the most likely scenario for human exposure, remains unknown. Utilizing C3H/HeN mice, transmission and dissemination kinetics of rickettsiae by a single *R. parkeri*-infected tick will be assessed. Ticks will be allowed to feed on hosts for 1, 6, 18, 48, and 144 hours. At the each time point, skin at the tick-bit site will be collected to quantify bacterial load. Whole blood and internal tissues, including the heart, liver, spleen, lung, and kidney, will be collected to assess bacterial dissemination. Furthermore, the expression of the inflammatory cytokines over the course of tick feeding will be evaluated.

Aedes aegypti salivary protein as an inhibitor of IFN-I signaling in human dermal fibroblasts

Meagan Taylor and Jonathan Rayner

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Chikungunya virus (CHIKV), an *Aedes* mosquito-borne virus, infects over 100,000 people each year and threatens approximately 1.3 billion worldwide. As climate change and rapid urbanization continue, *Aedes* species mosquitoes are expected to adapt to wider environments, creating new mosquito habitats and increasing virus transmission. Previous studies by other investigators found that *Ae. aegypti* saliva significantly increases the replication capacity of CHIKV and dengue virus (DENV) in human skin cells. For DENV, increased viral replication was attributed to specific salivary components, such as the putative 34kDa *Aedes* salivary protein (AaSG34), which decreased the capacity of mammalian cells to produce antiviral type I interferon (IFN-I). The specific IFN-I induction pathways impacted by AaSG34 and its contribution to CHIKV infection, which is known to induce a significant IFN-I response in mammalian cells, have not been elucidated. Based on this and other data in the literature, we hypothesize that the AaSG34 salivary protein inhibits RIG-I-dependent IFN-I signaling in mammalian cells, resulting in decreased IFN-I expression and increased CHIKV titers. To evaluate the capacity of AaSG34 to inhibit IFN-I expression and signaling, we treated human dermal fibroblasts with poly(I:C)/LyoVec in the presence of increasing concentrations of AaSG34 and measured transcript levels of IFN-I genes. Future studies will evaluate the impact of AaSG34 on CHIKV titers and IFN-I expression in human dermal fibroblasts, as well as uncover the pathogen recognition receptor impeded by AaSG34.

FPR-mediated signaling rescues neutrophil dysfunction in mut-STAT3 mice, promoting tissue repair in the lung

Killian Brewer and Robert Barrington

Department of Microbiology and Immunology, Frederick P. Whiddon College of Medicine, University of South Alabama, Mobile, AL, United States

Autosomal Dominant Hyper-IgE Syndrome (AD-HIES) is a rare, sporadically fatal primary immunodeficiency in children caused by autosomal dominant mutations in the signal transducer and activator of transcription 3 (STAT3) gene. AD-HIES patients exhibit recurrent lung infections facilitated by impaired recruitment of neutrophils. Impaired neutrophil chemotaxis to the infection site contributes to delayed epithelial resolution, lung damage, and patient mortality. While there is no cure for AD-HIES, the standard of care for recurrent lung infections in patients is prophylactic antibiotics administered over an extended period, thereby increasing the risk of antibiotic resistance without addressing impaired wound healing common in patients. Thus, there is a critical need for new therapeutic strategies that regulate neutrophil function to reduce recurrent infections and facilitate tissue repair in the lung. Our study aims to expand on current knowledge of neutrophil regulation and function to identify therapies that reduce recurrent infections and facilitate tissue repair. Neutrophils utilize Pattern Recognition Receptors (PRRs) to recognize and respond to pathogens. Among these, Formyl peptide receptors (FPRs) are a type of PRR that can recognize both foreign and self-ligands, playing a crucial role in both pro- and anti-inflammatory settings. Preliminary data from our lab shows that treatment with a bioengineered formyl peptide ligand enhances the chemotaxis of immunophenotypically distinct populations of neutrophils, akin to recently identified reparative neutrophils, suggesting the possibility of targeting specific ligands to modulate neutrophil function. We hypothesize that FPR-mediated signaling rescues neutrophil dysfunction in mut-STAT3 mice, a pre-clinical model of AD-HIES, promoting tissue repair in the lung. Our study aims to determine the mechanism of FPR-mediated rescue of mut-STAT3 neutrophil activity and determine the reparative capabilities of the recruited neutrophil population during active lung infection. This research endeavors to advance our understanding of neutrophil function and regulation, with the ultimate goal of improving treatments for AD-HIES and other immunodeficiencies characterized by neutrophil dysfunction.

Tissue localization and profiling of bacterial symbionts in the invasive Asian Longhorned Tick, *Haemaphysalis longicornis*

Shovon Lal Sarkar and Kevin Macaluso

Department of Microbiology and Immunology, Frederick P. Whiddon College of Medicine, University of South Alabama, Mobile, AL, United States

The Asian Longhorned Tick, *Haemaphysalis longicornis*, is naive to eastern Asia but was first detected in the United States in 2017. Established populations of *H. longicornis* have since been detected in 18 states, and ecological niche models predict that the species will continue to expand its geographic range in North America. Intriguingly, all *H. longicornis* collected in North America are morphologically female and reproduce by parthenogenesis. Our group and others demonstrated that this invasive tick is capable of acquiring and transmitting tick-borne pathogens endemic to North America, including *Rickettsia rickettsii*, Powassan virus, and Heartland virus. In addition to pathogens, ticks harbor a broad range of commensal microorganisms. Thus, the objective of this project was tissue localization and determination of major symbionts in a laboratory colony of *H. longicornis.* High-throughput sequencing of the V3-V4 hypervariable regions of the 16S rRNA gene was performed to investigate bacterial abundance and diversity in adult female H. longicornis. The bacterial population structure was assessed in individual *H. longicornis* females statically incubated at different temperatures (7, 22, and 31°C). Across the different temperatures, the whole *H. longicornis* female ticks exhibited varying bacterial diversity at 22°C, with the *Coxiella* genus having the highest relative abundance in all temperatures. 16S rRNA sequencing of salivary glands, midgut, and ovaries from *H. longicornis* females also showed a diverse bacterial population, with the salivary glands having the highest bacterial diversity of all organs. Together, these findings will lay the foundation for future studies examining the interactions between *H. longicornis*, commensal microbes, and pathogens.

Amyloid-beta regulates the innate immune response to pneumonia

Amanda N. Tuckey[1,2] and Jonathon P. Audia[1,2]

[1]Department of Microbiology and Immunology, Frederick P. Whiddon College of Medicine, University of South Alabama, Mobile, AL, United States
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While first described as an underlying driver of Alzheimer's disease pathology, amyloid-β (Aβ) has recently emerged as an antimicrobial peptide that is produced in response to infection. Indeed, Aβ is a pleiotropic innate immune effector that may function both as an initiator of the host response to infection and as a downstream activator of deleterious inflammation. Using our *Pseudomonas aeruginosa*-induced pneumonia mouse model, we discovered that mice lacking the ability to produce Aβ had higher mortality, higher bacterial burdens, and displayed reduced neutrophil infiltration into the lung. Furthermore, exposure of cultured pulmonary microvascular endothelial cells to Aβ initiated increased surface ICAM-1 expression, a critical component to neutrophil extravasation into tissues. Interestingly, Aβ is also a known activator of the NLRP3-caspase-1 inflammasome, and in silico analysis identified a putative caspase-1 cleavage site within Aβ. To demonstrate a possible novel regulatory relationship between these two innate immune effectors, we show that caspase-1 prevented spontaneous Aβ fibril formation in vitro. Thus, our vertically integrated animal, cell culture, and in vitro models highlight Aβ as a key regulator in the innate immune response to pneumonia. In an effort to extend these observations to humans, we undertook a study using plasma collected from intensive care unit (ICU) patients with sepsis, non-septic ICU patients, and healthy controls. Plasma Aβ levels were significantly elevated in sepsis patients and strongly correlated with indices of organ injury, independent of infection origination. Future studies will elucidate mechanisms underlying the innate immune role of Aβ in pneumonia and the effects of caspase-1 cleavage on Aβ antimicrobial activity.

Increased expression of Amyloid-β exhibits protective role against influenza A virus infection in a mouse model

Steven Smith and Jin Hyun Kim

Department of Microbiology and Immunology, Frederick P. Whiddon College of Medicine, University of South Alabama, Mobile, AL, United States

Alzheimer's disease (AD), the leading cause of dementia, is characterized by a progressive decline in cognitive function, yet the exact cause remains elusive despite extensive research. A hallmark feature of AD is the accumulation of amyloid-β (Aβ) plaques in the brain, which are closely linked to neurodegeneration, potentially contributing to memory loss. Emerging data suggest that the Aβ protein is capable of interfering with viral, bacterial, and fungal infections. Interestingly, recent biobank studies indicate that influenza and pneumonia are linked to several neurodegenerative diseases, but the protective role of Aβ against influenza infection is not known. In this study, we sought to determine if Aβ will mitigate replication of influenza A virus (IAV), thereby conferring improved clinical progression on infected mice. We tested this hypothesis by utilizing bioluminescent IAV to infect transgenic mice expressing human Amyloid Precursor Protein (hAPP), which contain familial AD mutations and can induce increased Aβ expression. We found that hAPP mice indeed had decreased viral replication compared to WT mice. Furthermore, hAPP mice had decreased loss in body weight and increased survival rate. Together, these data suggest that Aβ does have a protective role against IAV infection in the setting of our animal model.

Interspecies co-feeding transmission of Heartland virus between a native tick species, *Amblyomma americanum*, and the invasive East Asian Tick, *Haemaphysalis longicornis*

Parker Norman and Meghan Hermance

Department of Microbiology and Immunology, Frederick P. Whiddon College of Medicine, University of South Alabama, Mobile, AL, United States

The Asian Longhorned Tick, *Haemaphysalis longicornis*, is an invasive species from East Asia. Its population and geographical range are currently expanding in the United States. In a laboratory setting, *H. longicornis* has been shown to transmit an emerging North American tick-borne bandavirus, Heartland virus (HRTV). HRTV is responsible for human cases in the midwestern, northeastern, and southern United States. The main vector species for HRTV is the native *Amblyomma americanum* tick, which shares overlapping geographic territory with the invasive *H. longicornis* tick. In recent field studies, *H. longicornis* have been detected feeding alongside native *A. americanum* ticks on the same host animals. Consequently, we hypothesize that *H. longicornis* ticks co-feeding with native HRTV-infected *A. americanum* can acquire virus independent of host viremia. Using an in vivo tick transmission model, we tested our hypothesis by co-feeding HRTV-infected *A. americanum* nymphs with uninfected *H. longicornis* larvae or nymphs and screened the fed *H. longicornis* ticks for the presence of HRTV at different co-feeding proximities on the host. Using q-RT-PCR, HRTV RNA was detected in fed *H. longicornis* larvae and nymphs collected from multiple mice, providing evidence of interspecies co-feeding transmission of HRTV. Interestingly, this interspecies co-feeding transmission occurs in the absence of host viremia; therefore, it is possible that a localized skin infection facilitates HRTV transmission between co-feeding ticks in the absence of host viremia. Experiments are underway to further examine the role of host skin in co-feeding transmission of HRTV.

Minimum feeding time required for *Haemaphysalis longicornis* to transmit Severe Fever with Thrombocytopenia Syndrome Virus

Bailey Hettinger and Meghan Hermance

Department of Microbiology and Immunology, Frederick P. Whiddon College of Medicine, University of South Alabama, Mobile, AL, United States

Severe Fever with Thrombocytopenia Syndrome Virus (SFTSV) is an emerging tick-borne bunyavirus that can cause disease in humans with a 6-30% mortality rate. The major tick vector of SFTSV is *Haemaphysalis longicornis*, which is native to eastern Asia but has recently emerged in the United States. Emerging viruses such as SFTSV and invasive tick species such as *H. longicornis* highlight the importance of improving our understanding of how tick-borne viruses are maintained and transmitted by ticks. The minimum time required for a tick to transmit pathogens to a host is understood for some bacteria but for tick-borne viruses, particularly SFTSV, this tick-to-host transmission time is unknown. The objective of this study is to define the minimum feeding time required for an *H. longicornis* nymph to transmit SFTSV to a host. Two groups of SFTSV-infected *H. longicornis* nymphs will be generated and compared: one will be infected by feeding on a viremic host as larvae, and the other will be infected by transovarial transmission. In comparing the two groups of SFTSV-infected nymphs, the best fit model will be the group with the highest levels of SFTSV viral RNA. The best fit model of these two nymph groups will be used to then determine the minimum feeding time required for an *H. longicornis* nymph to transmit viable SFTSV to a mouse host. All samples will be processed by RNA extraction and screened via q-RT-PCR. Samples will also be screened for infectious SFTSV using a Focus Forming Assay.