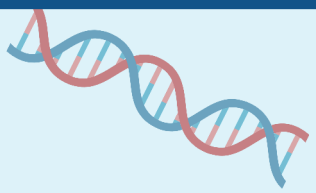
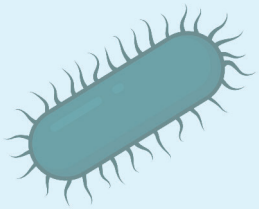
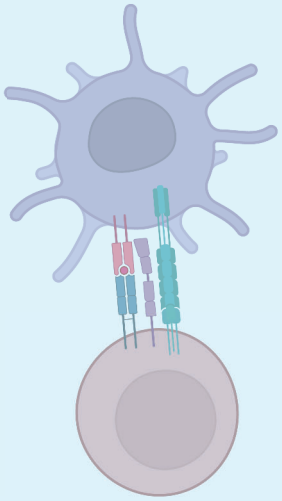




Microbiology and Immunology

Research Symposium

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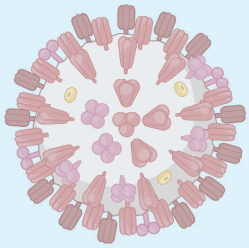
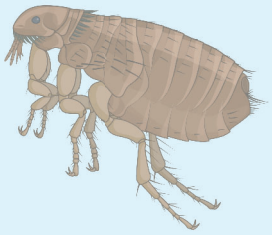
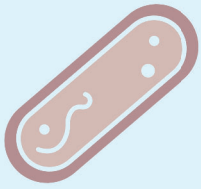
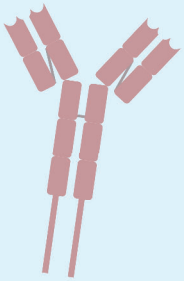

August 14, 2025

August 14th, 2025

Oakleigh Room at USA Technology & Research Park

2025 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 "The path to uncovering an immune conversation that predicts preterm birth" by Dr. Kathryn Patras, Molecular Virology and Microbiology Assistant Professor, Baylor College of Medicine in Houston, TX
1:00 PM	Intermission
1:15 PM	Student Research Presentations begin. *First Presentation*—Hoa Tran *Second Presentation*—Meagan Taylor *Third Presentation*—Olu Adesunloro *Fourth Presentation*—Brianna Mitchell *Fifth Presentation*—Killian Brewer
2:30 PM	Intermission.
2:45 PM	Student Research Presentations resume. *Sixth Presentation*—Shovon Lal Sarkar *Seventh Presentation*—Sarah Macon-Foley *Eighth Presentation*—Nam Suwanbongkot *Ninth Presentation*—Manley Hicks
3:45 PM	The 2025 IDHD Symposium concludes. Dr. Shea gives closing remarks. Meet the Track presentation facilitated by Dr. Meghan Hermance, Whiddon COM Microbiology and Immunology Associate Professor and IDHD Track Director, begins.
4:00 PM	Meet the Track presentation concludes.

Pseudomonas aeruginosa Type III secretion system effector ExoU activates caspase-1, thereby triggering programmed cell death in lung endothelial cells

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ExoU is a critical virulence factor of *Pseudomonas aeruginosa* that is injected into host cells by the Type III secretion system. Beyond its phospholipase-driven cytotoxicity, ExoU-expressing strains also activate caspase-1 and induce pyroptosis—a programmed cell death (PCD) pathway regulated by caspase-1/11 (or caspase-4/5 in humans). Canonical pyroptosis involves inflammasome assembly, caspase-1 activation, and GSDMD-mediated pore formation, leading to cell lysis and release of IL-1β, IL-18, and lactate dehydrogenase (LDH). Considering that much of the published work focused on pyroptosis in macrophages as a model immune cell, we sought to examine the effects of ExoU on caspase-1 activation in our established pulmonary microvascular endothelial cell (PMVEC) infection model with *P. aeruginosa* strain PA103 (encoding ExoU and ExoT).

To explore the relationship between ExoU, caspase-1, and pyroptosis, we generated bi-allelic knockouts of *Casp1* and *Gsdmd* in PMVECs using a CRISPR-Cas9 approach. In this model, we compared PA103 to isogenic mutants lacking ExoU and/or ExoT. An LDH release-based cytotoxicity assay revealed that *Casp1*-deficient PMVECs exhibit a substantial delay in LDH release in response to infection with PA103 strains expressing ExoU when compared to wild-type PMVECs. These data suggest that ExoU requires caspase-1 to initiate an early PCD response. Strikingly, the time course of *Gsdmd*-deficient PMVECs lysis in response to infection with PA103 strains expressing ExoU was not different than wild type PMVECs. These data suggest the ExoU-caspase-1-regulated PCD pathway is independent of GSDMD. We are currently investigating whether ExoU engages alternative PCD pathways, which would represent a novel virulence mechanism of *P. aeruginosa*.

Using primary human dermal fibroblasts as an *in vitro* model of chikungunya virus infection at the vector-virus-host interface

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. Despite its global burden and transmission by *Aedes* mosquitoes, the role of mosquito saliva in CHIKV infection remains poorly understood. Further confounding this understanding is the lack of an adequate *in vitro* system for studying early CHIKV infection dynamics. Here, we introduce such a model using primary human dermal fibroblasts (hDFs), one of the most abundant cell types at the vector-virus-host interface, to explore CHIKV infection dynamics. Using hDFs from two different donors, we tracked differences in the accumulation of infectious titers over time using TCID50, as well as IFNβ transcript expression as a marker for innate immunity using RTqPCR. Additional variables evaluated included the CHIKV strain, source of the virus stocks, and multiplicity of infection (MOI). We found that hDFs are highly permissive to CHIKV infection and exhibit extensive cell death, regardless of the variables assessed. Additionally, the starting MOI significantly influenced IFNβ transcript levels, which has substantial implications for using hDFs as a model to evaluate mosquito saliva-assisted transmission. These findings underscore the potential of our model for investigating the impact of mosquito saliva on early CHIKV infection and the resulting innate immune response, promising to fill a critical research gap in understanding virus-host interactions at the initial point of contact.

Importance of manganese uptake in uropathogenic *Escherichia coli* CFT073 during urinary tract infection

Olu Adesunloro and Allyson Shea

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Urinary tract infection (UTI), the second most common bacterial infection worldwide, occurs when bacteria normally residing in the gut colonize the bladder, causing cystitis. If left untreated, the infection can ascend to the kidneys, leading to pyelonephritis and, in severe cases, progressing to urosepsis. *Uropathogenic Escherichia coli* (UPEC) is the primary causative agent of UTIs and relies on iron and other cationic metals to support its metabolic functions. In response, the host restricts metal availability as part of its nutritional immunity during infection. To understand how UPEC overcomes this restriction, we investigated the roles of the Sit, Feo, and Efe iron transport systems in UPEC strain CFT073 during infection. Deletion of sit, but not feo or efe, resulted in significant fitness defects in both urine and kidney tissues in a murine model of UTI ($p < 0.05$). We hypothesized that the *in vivo* defect of the Δsit mutant may be due to its additional role in manganese transport, unlike Feo and Efe, which exclusively transport iron. Consistent with this, deletion of either sit alone or both sit and mntH (another manganese transporter) caused a growth defect in metal cation-limited minimal medium compared to the wild-type strain. Strikingly, loss of both Sit and MntH led to a profound loss of fitness *in vivo*. Together, these findings support a model in which manganese uptake is critical for UPEC colonization in the iron-restricted environment of the host.

Mechanisms of cytokine release by monoclonal antibody therapies

Brianna Mitchell and Michael R. Elliott

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Monoclonal antibodies (mAbs) are widely used to treat cancer, autoimmune diseases, and infectious diseases. However, mAb administration in treatment-naïve patients frequently triggers a rapid spike in cytokine levels in the blood often resulting in cytokine release syndrome (CRS). In a subset of patients, CRS is associated with rapid onset of fever, hypotension, rash, chills, and dyspnea, termed first-dose infusion reaction (FDIR). For example, first administration of mAb therapies for chronic lymphocytic leukemia (CLL) patients results in 64% with CRS of which 77% have FDIRs. Even at low doses, rituximab, an αCD20 mAb for treating CLL, causes rapid elevation of many inflammatory mediators in circulation (e.g. IL-6, IL-8, CXCL10). Despite this, the mechanism of cytokine release by αCD20 mAbs is unknown. Current findings show within minutes after αCD20 infusion, circulating B cells are opsonized by mAbs and rapidly engulfed by liver and spleen macrophages through antibody-dependent cellular phagocytosis (ADCP), the key cytotoxic mechanism of αCD20 mAbs. Rapid onset of ADCP following initial αCD20 infusion correlates with CRS, suggesting ADCP by tissue-resident macrophages may contribute to αCD20-induced cytokine release. Our preliminary studies of human monocyte-derived macrophages (hMDMs) co-cultured with αCD20-opsonized CLL cells demonstrated significant increases of inflammatory cytokines IL-6, IL-8, and CXCL10 in cell supernatants, supporting a model in which cytokine release is directly linked to macrophage ADCP. Thus, we hypothesize αCD20-mediated ADCP causes macrophages to release inflammatory cytokines that contribute to CRS. We will test this using *in vitro* and *in vivo* approaches with αCD20 mAbs in the context of CLL.

Neutrophil hyperresponsiveness contributes to lung pathology in *STAT3*$^{V463\Delta}$ mice

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Autosomal dominant hyper-IgE syndrome (AD-HIES), or Job's syndrome, is a rare primary immunodeficiency caused by dominant-negative mutations in *STAT3*. Patients experience recurrent pulmonary infections and chronic inflammation, leading to severe complications and heightened mortality risk. To investigate whether neutrophil-intrinsic dysfunction contributes to lung pathology in AD-HIES, we used a murine model expressing the *STAT3*$^{V463\Delta}$ mutation, a common disease-associated variant. Following intratracheal infection with *Pseudomonas aeruginosa*, *STAT3*$^{V463\Delta}$ mice exhibited pronounced alveolar damage, increased vascular congestion, and extensive leukocyte infiltration compared to wild-type (WT) controls. These changes were accompanied by elevated bacterial burden and significantly increased levels of pro-inflammatory cytokines and chemokines in the lung. Neutrophil recruitment to the lungs was markedly elevated, and surface expression of degranulation markers was enhanced in *STAT3*$^{V463\Delta}$ neutrophils *in vivo*. To determine whether neutrophil hyperactivation was driven by intrinsic defects independent of microbial load, mice were challenged intratracheally with purified lipopolysaccharide (LPS), revealing similarly enhanced neutrophil degranulation in *STAT3*$^{V463\Delta}$ mice despite controlled PAMP exposure. Mechanistically, bone marrow–derived neutrophils (BMDNs) from *STAT3*$^{V463\Delta}$ mice displayed heightened degranulation and NETosis in response to PMA or f-MLF stimulation. Together, these findings demonstrate that *STAT3*$^{V463\Delta}$ drives neutrophil hyperresponsiveness, contributing to dysregulated inflammation and pulmonary tissue damage in AD-HIES.

Characterization of *Amblyomma maculatum* saliva microRNAs and their role in *Rickettsia* transmission

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Amblyomma maculatum, known as the Gulf coast tick, is an aggressive tick that bites both animals and humans. The tick harbors *Rickettsia parkeri*, a human pathogen that causes spotted fever group rickettsiosis, and *Candidatus* Rickettsia andeanae, which is considered non-pathogenic to humans. Gulf Coast ticks transmit both rickettsial agents through saliva during blood-feeding. Recent studies on salivary miRNA profiles in tick species suggest that differentially expressed miRNAs may influence tick development, feeding activity, and pathogen transmission. This research aims to create a complete profile of the saliva miRNA *in A. maculatum* infected with *R. parkeri* or *Ca.* R. andeanae, and to functionally characterize selected differentially expressed miRNAs through RNA interference (RNAi) assays. To investigate the roles of tick miRNAs in rickettsial transmission and related pathology, the experimental focus of this study is to compare miRNA expression in the saliva of partially fed, female, uninfected ticks with that of ticks infected with pathogenic and non-pathogenic *Rickettsia*. Sequencing saliva miRNA has allowed for the prediction of function for differentially expressed miRNAs through *in silico* target analysis. Preliminary data indicate that *R. parkeri*-infected tick saliva has a different miRNA profile than uninfected saliva, with most miRNAs downregulated in infected ticks. *In silico* target prediction for miRNAs and RNAi assays in ticks targeting specific miRNAs using miRNA mimics (AgomiR) or anti-miRNAs (AntagomiR) will aid in identifying the roles of differentially expressed miRNAs.

Tick co-feeding transmission of tick-borne flaviviruses: Developing a working model system

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Tick co-feeding transmission occurs when infected ticks pass virus to uninfected ticks simultaneously feeding on the same vertebrate host. Recent studies in our lab indicate that a localized skin infection appears to facilitate tick co-feeding transmission of flaviviruses. Previous research demonstrated that immune cells emigrating from the infected tick (i.e. "donor") feeding site contain viral antigen, suggesting that tick feeding recruits virus-infected cells from the feeding site of donor ticks to pathogen-free "recipient" ticks that are co-feeding on the same vertebrate host. Although co-feeding transmission has been shown for a variety of tick-borne viruses, the underlying cellular and molecular mechanisms responsible for this route of virus transmission between co-feeding ticks remain undefined. To address this gap, we aim to use a model system involving Langat virus, *Ixodes scapularis*, and a murine vertebrate host to study co-feeding transmission of tick-borne flaviviruses. Our initial work demonstrated co-feeding transmission of LGTV between infected donor ticks and initially uninfected recipient ticks on mice that developed a detectable viremia. Given that for many tick-borne viruses, viremia, in various species of wild animals, is either transient and low in titer or entirely absent, we are working to further refine our model to eliminate host viremia during tick co-feeding. Ultimately, understanding the mechanisms underlying co-feeding transmission will reveal novel targets for preventing tick-borne virus transmission and provide insights into the enzootic maintenance of these viruses in nature.

Cutaneous response to rickettsial infection via a single infected tick feeding events

Nam Suwanbongkot and Kevin Macaluso

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Frederick P. Whiddon College of Medicine, Mobile, AL, United States

Rickettsia parkeri, an emerging bacterial pathogen, is transmitted via infected tick saliva. During feeding, ticks secrete numerous salivary factors manipulating the host's hemostatic and immune response to promote blood feeding and facilitating pathogen transmission. Previous studies have examined the host immune response to *Rickettsia* via needle inoculation or uninfected tick feeding. However, the interactions occurring at the skin interface during tick-mediated transmission remain poorly understood. In this study, we will elucidate the cutaneous immune response to rickettsial infection delivered by the tick vector. Utilize a murine model and nature tick vector, *Amblyomma maculatum*, to assess the host response to *R. parkeri* infection inoculated by the tick. Using a single tick model, an uninfected or *R. parkeri*-infected tick was fed on C3H/HeN mice for various timepoints. Skin biopsies at the tick feeding site were collected to measure cytokine response and cellular infiltrate at the tick attachment site by flow cytometry. In response to feeding by *R. parkeri*-infected ticks, a significant increase in concentrations of IFN-γ, TNF-α, IL-6, IL-4, MPC-1, and GM-CSF was observed at the late time point (6 days post tick attachment, dpa) in the infected tick feeding group. Conversely, levels of IL-1β exhibited a marked decrease at all time points in comparison to naïve mice. Additionally, at 6 dpa, a comparison of uninfected tick feeding revealed an increase in the levels of IFN-γ, TNF-α, IL-6, IL-4, MPC-1, and GM-CSF in the *R. parkeri*-infected group. Furthermore, enhanced cellular infiltration was observed at the tick attachment sites in the *R. parkeri*-infected group compared to the uninfected group at all time points. These results indicate the importance of host responses elicited specifically by tick-mediated pathogen transmission, providing a deeper understanding of the host-pathogen interaction dynamics at the cutaneous level.

Optimizing macrophage phagocytosis in monoclonal antibody therapies

Manley Hicks and Michael R. Elliott

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Monoclonal antibody (mAb) therapies have revolutionized cancer treatment by enabling targeted elimination of malignant cells. However, their efficacy is constrained by dependence on the finite cytotoxic capacity of the patient's immune system – particularly effector mechanisms like antibody-dependent cellular phagocytosis (ADCP). ADCP is primarily executed by macrophages which engulf and clear mAb-opsonized cancer cells. We recently discovered this process is impaired by macrophage hypophagia, a dysfunctional state marked by reduced phagocytic activity. This immune exhaustion limits mAb-mediated tumor cell clearance and contributes to therapeutic resistance. In chronic lymphocytic leukemia (CLL) – a B cell malignancy commonly treated with anti-CD20 mAbs like rituximab or obinutuzumab – such resistance is a well-known clinical challenge. Although these agents initially deplete malignant B cells effectively, subsequent treatments are much less effective, limiting the efficacy of anti-CD20 as a monotherapy. Our lab has identified a regulatory imbalance driving this phenomenon: a decrease in pro-phagocytic Fc gamma receptors (FcγRs) alongside an increase in anti-phagocytic adenosine receptors (A2Rs). FcγRs are essential for macrophage recognition and engulfment of mAb-opsonized targets. We have shown that FcγR-mediated phagocytosis induces receptor internalization, leading to sustained reduction in surface FcγRs and suppression of ADCP. Concurrently, A2Rs – which elevate intracellular cAMP, a potent inhibitor of phagocytosis – are upregulated, compounding inhibition of macrophage function. Thus, our central hypothesis is that macrophage hypophagia results from both reduced FcγR expression and signaling downstream of FcγRs. To test this, we will employ inducible FcγR overexpression systems and A2R modulation in primary mouse macrophages, using ADCP assays to evaluate functional restoration.