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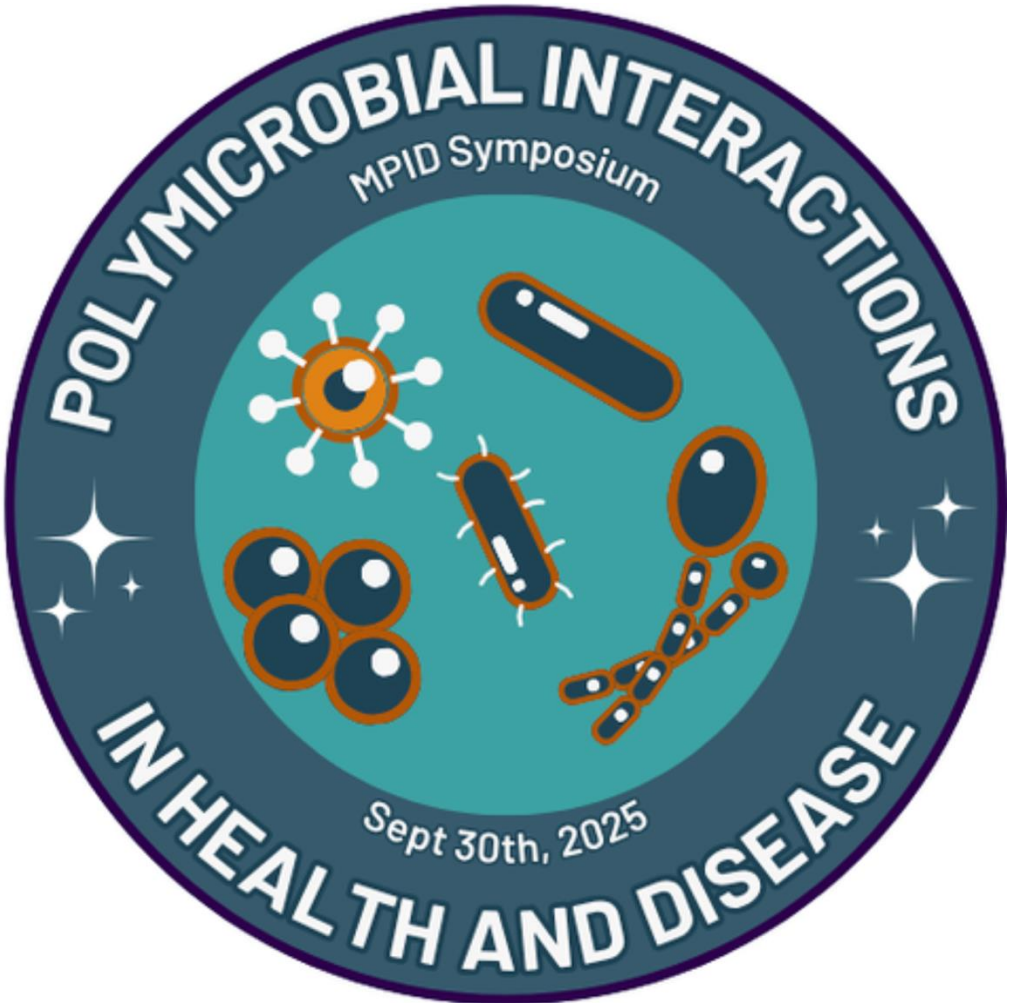
Anschutz Medical Campus

Department of Immunology & Microbiology



Tuesday, September 30, 2025
Krugman Hall

Molecular Pathogenesis of Infectious Disease Symposium

Polymicrobial Interactions in Health and Disease

Tuesday, September 30, 2025

Krugman Hall

Schedule

Time	Event
8:30 – 8:55 AM	Registration and Coffee
8:55 – 9:00 AM	Opening Remarks
9:00 – 9:30 AM	**Jessica Metcalf, PhD** *CSI Microbe: Big Clues from Tiny Witnesses*
9:30 – 10:00 AM	**Natalie Lamb, PhD** *Secure biosystems design in Saccharomyces cerevisiae establishes effective biocontainment strategies and mechanisms of escape*
10:00 – 10:15 AM	Break
10:15 – 10:45 AM	**Mike Schurr, PhD** *Acrylated Hydroxyazobenzenes to Suppress Streptococcus species in Oral Microbiomes*
10:45 – 11:45 AM	**R. Balfour Sartor, MD** Intestinal microbial, environmental, and immune interactions: What is known and where we need to venture.
11:45 – 1:30 PM	Lunch
1:30 – 3:30 PM	Poster/Vendor Session
3:45 – 4:00 PM	Poster Awards

Speakers

Jessica Metcalf, PhD
Professor
Department of Animal Sciences
Colorado State University

Jessica Metcalf is a Professor in the Department of Animal Sciences and the Associate Director of the Cell & Molecular Biology Graduate Program at Colorado State University. She earned her Ph.D. in Ecology and Evolutionary Biology from the University of Colorado and completed postdoctoral training at the Australian Centre for Ancient DNA and in Dr. Rob Knight's lab at CU Boulder before establishing her own lab at CSU. Her work bridges vertebrate evolution, microbial ecology, human health, and forensic science, with a particular focus on how microbial communities shift across hosts, environments, and time. Dr. Metcalf's research has been featured in popular media, including Ed Yong's *I Contain Multitudes* series, NPR's *Science Friday*, *Newsweek*, *The Atlantic*, and *The New York Times Magazine*.

Natalie Lamb, PhD
Postdoctoral Researcher
National Renewable Energy Laboratory (NREL)

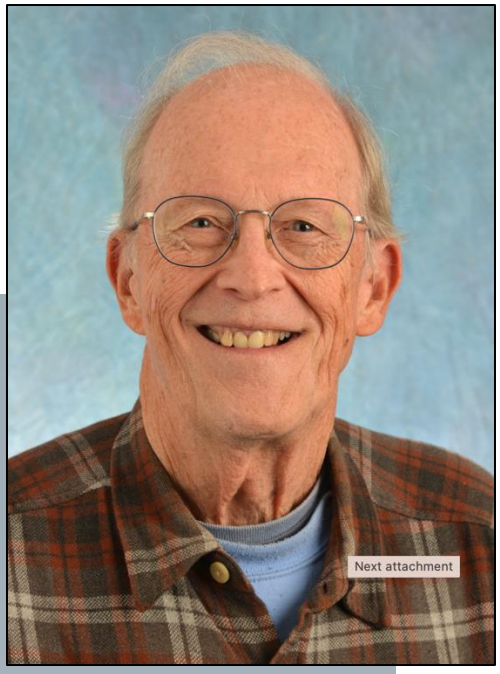
Natalie Lamb is a researcher specializing in developing synthetic biology approaches in the budding yeast *Saccharomyces cerevisiae*. She completed her PhD studying DNA metabolism and genomic stability in budding yeast at the University at Buffalo in New York state. She went on to work as a bioinformatician in the University of Buffalo Genomics and Bioinformatics core before starting her postdoctoral training at NREL, where she is developing effective biocontainment strategies that are broadly applicable to the different domains of microorganisms.

Speakers

Michael Schurr, PhD
Professor
Department of Immunology & Microbiology University of Colorado Anschutz School of Medicine

Dr. Michael J. Schurr is a Professor at the University of Colorado School of Medicine, where he leads research on the pathogenic mechanisms of *Pseudomonas aeruginosa* in cystic fibrosis (CF). He also serves as President of Azodent, Inc., collaborating to develop innovative cavity coating materials. Dr. Schurr earned his Ph.D. in Molecular Biology from the University of North Texas and completed postdoctoral training at UT San Antonio. He has held faculty positions at the University of Michigan, Tulane, and has been at the University of Colorado since 2006. His lab's work advances both CF therapeutics and dental hygiene. Dr. Schurr serves on NIH grant panels and editorial boards and is a recipient of the Cystic Fibrosis Foundation's New Investigator Award.

Keynote Speaker



R. Balfour Sartor, MD
Midget Distinguished Professor of Medicine
Department of Medicine
University of North Carolina at Chapel Hill
School of Medicine

Dr. Balfour Sartor is a Midget Distinguished Professor of Medicine in the Division of Gastroenterology and Hepatology at the University of North Carolina at Chapel Hill. He also serves as Director of the National Gnotobiotic Rodent Resource Center and Co-Director of the Center for Gastrointestinal Biology and Disease, two internationally renowned cores that support cutting-edge research in host–microbiome interactions. He completed both his medical degree and residency at Baylor College of Medicine. Afterwards, he pursued a clinical and research fellowship in gastroenterology at UNC-Chapel Hill, where he established his research lab. He is a pioneer in host-microbiota interactions and mucosal immunology research, with his research emphasizing mechanisms by which resident bacterial subsets stimulate protective vs inflammatory mucosal immune responses. He has been honored with some of the most prestigious awards in his field. His sustained funding from the NIH for several years underscores the lasting impact and relevance of his work. Dr. Sartor has published over 400 articles/book chapters/editorials, edited books, and has trained many graduate students and post-doctoral fellows. His publications appear impactful journals such as Gastroenterology and Nature, where his contributions have innovated and shaped the field on how microbial communities and their interactions influence the host and modulate health and disease.

Abstracts

Poster #	Presenter	Title
1	Shelby Andersen	Discovery of antiphage defense systems using a conserved serine recombinase as bait
2	Lauren Atencio	Dual sensing of cellular stress and peptidoglycan by NOD2 in the Citrobacter rodentium-induced colitis model
3	Sofia Christensen	Identification of Scavenger Receptors as a Mechanism for DENV Clearance from Blood Circulation
4	Shirli Cohen	Candida albicans promotes Streptococcus agalactiae ascending infection through physical and metabolic interactions
5	Arianne Crossen	Contribution of Group B Streptococcal Adhesin BspC to Neonatal Intestinal Colonization and Interactions with Candida albicans
6	Erika Desonie	A Natural Product Derivative Increases Bacteriophage Infectivity
7	Ana Fairbanks-Mahnke	Protective Roles of Airway Prevotella in Epithelial Cell Activation and Defense Against Pneumococcal Pneumonia
8	Pedro Gamez	Developing a Model System to Advance Treatments for Gammaherpesvirus-Associated Lymphomas
9	Sydney Hall	Characterizing Staphylococcus aureus oxoproline metabolism and role in skin infection
10	Luke Hanson	Strain-dependent expression of an immunogenic adhesin-like protein that confers a colonization advantage in the gut

Abstracts

Poster #	Presenter	Title
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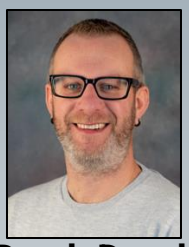
12	Alyx Job	Interactions between Group B Streptococcus and Enterococcus faecalis in the murine diabetic wound
13	Sophia Kim	Meningeal cell contribution to barrier breakdown during neonatal bacterial meningitis
14	Rachael Kostelecky	Divergent outcomes of MHV68 infection in myeloid cells implicate cytokine signaling in lytic restriction
15	Anna-Sophia Leon	Mechanisms of Staphylococcus aureus resistance to commensal antimicrobial peptides
16	Nicole Messner	Role of cardiolipin synthase in daptomycin resistant Enterococcus faecium
17	Elizabeth Nail	IRE1 and NOD2 Activation Confers Protection Against Salmonella Typhimurium Dissemination
18	Gracyn Nelson-Reid	Analysis of in vitro and in vivo macrophage infection by murine gammaherpesvirus 68
19	Steven Shaw	Type I Interferon signaling enhances Streptococcus pneumoniae middle ear infection
20	Elizabeth Spear	The structural basis for coordination between tandem exonuclease resistant RNAs (xrRNAs) in dengue virus
21	Emma Trujillo	Investigating the Role of Biofilm Formation as a Driver for Candida albicans-associated inflammation in IBD

Acknowledgements

Department of and Microbiology immunology Leadership

University of Colorado Anschutz School of Medicine

Dr. Linda van Dyk
Professor
Vice Chair



Dr. Breck Duerkop
Associate Professor
Co-Director, Microbiology Program

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Discovery of antiphage defense systems using a conserved serine recombinase as bait

Shelby Andersen

Poster # 1

With limited treatment options available for Multi-Drug Resistant (MDR) infections, bacteriophage (phage) therapy has re-emerged as a potential treatment option. Antiphage systems that help bacteria evade phage infection threaten the efficacy of phage therapy. We recently characterized a Type IV restriction enzyme in Enterococcus faecalis that is co-transcribed with a predicted serine recombinase. Herein, we developed a bioinformatic pipeline that uses this serine recombinase as bait to identify potential antiphage systems, which has been termed Recombinase Associated Defense Search (RADS). Using RADS to query reference genomes and metagenomic contigs, we discovered known antiphage defense systems that are genetically linked to serine recombinase genes. Using co-transcriptional and binomial analyses to prioritize the testing of genes, we discovered novel antiphage systems and offer preliminary characterization of two such systems. First, we reveal that KAP NTPase domains regulate diverse antiphage effectors. Second, we demonstrate that Schlafen proteins are antivirals that are conserved across the tree of life. RADS serves as an unbiased method for identifying antiphage systems across bacterial phyla.

Dual sensing of cellular stress and peptidoglycan by NOD2 in the Citrobacter rodentium-induced colitis model

Lauren Atencio

Poster # 2

Citrobacter rodentium (Cr) is an enteric mouse pathogen that causes attaching and effacing lesions within the intestinal mucosa. These lesions are indistinguishable from those formed by enteropathogenic and enterohaemorrhagic E. coli, one of the main causes of foodborne disease. As such, the scientific community widely accepts the use of Cr as a model organism to study host responses to infection. Here, we use Cr to explore the pattern recognition receptor (PRR) NOD2. The intracellular PRR NOD2 is classically known to recognize the peptidoglycan fragment muramyl dipeptide (MDP) during infection and initiate a robust pro-inflammatory response. The most common mutation in NOD2 is a frame shift caused by a cytosine insertion, resulting in a truncated NOD2 lacking the last 33 AAs (NOD2m/m). NOD2m/m is unresponsive to stimulation with MDP, and it is believed that this 'loss-of- function' phenotype is the cause of an increased risk to develop inflammatory bowel disease (IBD). Previous work by the Keestra-Gounder lab has identified that NOD2 senses perturbations in endoplasmic reticulum homeostasis (ER stress). Here we demonstrate that NOD2m/m cannot sense MDP yet does sense ER stress signals. Little is known how NOD2m/m contributes to intestinal inflammation in the absence of peptidoglycan recognition. We therefore sought to examine the immune response initiated by NOD2m/m in the Cr-induced colitis model. We demonstrate that Nod2-/- mice have significantly delayed bacterial clearance compared to littermate controls (Nod2+/m), whereas Nod2m/m mice show an intermediately ckear Cr in vivo. We are currently investigating the differential immune responses over the course of infection that contributes to efficient clearance of Cr from the intestinal tract. Overall, our data suggests that NOD2m/m does not confer a complete loss-of-function and NOD2 sensing of both peptidoglycan and ER is required to induce an appropriate immune response to clear Cr from the intestinal tract.

Identification of Scavenger Receptors as a Mechanism for DENV Clearance from Blood Circulation

Sofia Christensen

Poster # 3

Dengue virus (DENV) is a positive sense, single-stranded RNA virus that causes more annual infections worldwide than any other arthropod-borne virus. This mosquito transmitted virus causes more than 500 million infections globally each year, making it a major global health concern. Severe DENV infection is associated with high viremia, which is in part determined by the host's ability to clear the virus from the bloodstream. Our prior studies demonstrated that Kupffer cell depleted mice have impaired DENV clearance from the bloodstream, indicating that Kupffer cells, the liver tissue resident macrophages, are involved in controlling viremia. Additional studies showed that blocking scavenger receptors using poly(I) and dextran sulfate limit the ability of mice to clear DENV. From the literature, we identified a panel of 4 scavenger receptors known to be expressed by Kupffer cells and to be inhibited by poly(I) and dextran sulfate: CD163 (SR-I1), SCARF1 (SR-F1), OLR1 (SR-E1), and COLEC12 (SR-A4). An additional scavenger receptor of interest is LRP1 (SR-L1), which is expressed by Kupffer cells and has been previously shown to interact with DENV. To determine if one or more of these scavenger receptors promote DENV binding and internalization, we are creating stable U937 cells that overexpress each individual scavenger receptor. U937 cells are human myeloid cells that are permissive but not susceptible to DENV infection unless they ectopically express a receptor that promotes virus internalization. Thus, we will use the panel of scavenger receptor expressing cell lines and our established DENV internalization assay to investigate whether these scavenger receptors mediate DENV infection in otherwise non-susceptible cells. This research will aid in identifying receptors involved in controlling DENV viremia, which ultimately could be targeted to reduce disease transmission and severity.

Candida albicans promotes Streptococcus agalactiae ascending infection through physical and metabolic interactions

Shirli Cohen

Poster # 4

The vaginal microbiome can include Group B Streptococcus (GBS), a pathobiont that is associated with adverse pregnancy outcomes and severe neonatal disease. Candida albicans (Ca) vaginal colonization is a risk factor for GBS carriage; however, our understanding of their association is limited. We have developed a murine model of Ca-GBS co-colonization and find that Ca promotes GBS persistence in the female genital tract. Ca and GBS form dense polymicrobial aggregates, protecting the bacteria from the environment and anchoring it to the vaginal epithelium. Transcriptomics analysis has revealed that co-colonization induces differential regulation of microbial adhesins and virulence factors. Additionally, GBS induces Ca arginine biosynthesis, which is directly implicated in GBS pathogenesis by upregulating factors that promote persistence in the host and adhesion to Ca. Future work will address how co-colonization may alter the host environment to be more permissive to GBS by reducing the inflammatory response.

Contribution of Group B Streptococcal Adhesin BspC to Neonatal Intestinal Colonization and Interactions with Candida albicans

Arianne Crossen

Poster # 5

Group B Streptococcus (GBS) is the leading cause of neonatal meningitis worldwide. Though GBS benignly colonizes the GI tract in 30% of healthy adults, colonization of the neonatal GI tract can lead to serious invasive disease. The mechanisms by which GBS colonizes the intestinal tract are not well defined, and we aim to better characterize this step in disease progression. Surface-anchored antigen I/II family adhesins are a widespread virulence factor among streptococcal species, promoting bacterial adhesion to host cells. In GBS, Group B streptococcal surface protein C (BspC) is particularly associated with hypervirulent meningitis-associated sequence type 17 (ST-17) strains and has been shown to promote GBS adherence to vaginal epithelial cells. We hypothesize that BspC contributes to GBS' ability to colonize the intestinal tract. Preliminary data reveals that an isogenic bspC mutant in the ST-17 strain COH1 displayed significantly impaired adherence to both Caco-2 and primary human infant colonic epithelial cells. Mutations to the variable domain of BspC and treatment of Caco-2 cells with an antibody specific to the proposed BspC host receptor, cytokeratin 19, significantly reduced GBS adherence. In a neonatal murine meningitis model, mice infected with bspC survived significantly better than wildtype infected mice, and displayed reduced intestinal bacterial burden. Interestingly, BspC has been shown to contribute to GBS interactions with the Candida albicans, an opportunistic fungus that colonizes the neonatal intestinal tract. We have shown that the presence of C. albicans enhances GBS adherence to intestinal epithelial cells, and we next aim to determine whether coinfection with C. albicans enhances intestinal colonization in vivo. This work suggests that BspC contributes to GBS colonization of the neonatal intestinal tract through both direct interaction with intestinal epithelial cells and indirectly through interaction with C. albicans.

A Natural Product Derivative Increases Bacteriophage Infectivity

Erika Desonie

Poster # 6

The rise of multidrug resistant bacterial infections has led to investigations of alternative treatment options such as bacteriophage (phage) therapy. Small molecules, such as antibiotics and metabolites, can synergize with phages to efficiently kill bacteria. In many of these cases the mechanisms underlying these synergistic activities are unknown. For phages to become widely used therapeutics, we must understand how phages work in collaboration with small molecule adjuvants to support robust therapeutic potential. In this study, using phages that target Enterococcus faecalis in combination with compounds from a natural product library, we identify a steviol derivative that increases phage lysis at normally sublethal multiplicity of infection (MOI) through an unknown mechanism. Future work aims to identify the mechanistic basis of phage-steviol combinatorial killing activity. Elucidating this mechanism will benefit the future development of phage therapies to treat drug resistant bacterial infections.

Protective Roles of Airway Prevotella in Epithelial Cell Activation and Defense Against Pneumococcal Pneumonia

Ana Fairbanks-Mahnke

Poster # 7

The airway microbiome is an under-characterized niche of the human body, capable of both influencing states of health by priming and activating immune responses, as well as states of disease during dysbiosis. Certain members of the airway microbiome including Prevotella melaninogenica (P. mel.) have long been characterized as more abundant during states of health and are associated with decreased carriage of respiratory pathogens like Streptococcus pneumoniae, but a causative link has not been identified. S. pneumoniae is the leading cause of community acquired pneumonia, and the leading cause of infectious death in children worldwide. We found that P. mel. induces rapid clearance of S. pneumoniae from the lungs of mice in a neutrophil dependent manner via toll-like receptor (TLR)2 activation. Understanding the P. mel-derived signals that are necessary to recruit and activate neutrophils to the airway is paramount for further characterizing how P. mel. exposures protect against S. pneumoniae infections. Preliminary data from our group has demonstrated that airway epithelial cells become activated and secrete neutrophil recruiting and activating chemokines in response to P. mel. exposure. We have furthermore identified that S. pneumoniae adherence to P. mel.-exposed epithelial cells is decreased, impacting an essential step in S. pneumoniae pathogenesis. The major adherence receptor S. pneumoniae uses to adhere to epithelial cells is Platelet Activating Factor Receptor (PAFR/PTAFR) We have found that while P. mel. stimulation itself is not sufficient to decrease PTAFR mRNA, P. mel. stimulation decreased S. pneumoniae-induced upregulation of PTAFR in multiple in vitro models of S. pneumoniae infection. Further, inhibition of TLR2 during P. mel. exposure in cell culture results in decreased production of IL-8 and CXCL2, suggesting that TLR2 is important for the epithelial response to P. mel.. Together, these results suggest that P. melaninogenica activation of TLR2 ind

Developing a Model System to Advance Treatments for Gammaherpesvirus-Associated Lymphomas

Pedro Gamez

Poster # 8

Epstein-Barr virus (EBV) is a human gammaherpesvirus (yHV) that infects most of the human population. Most healthy individuals develop no signs of disease upon infection; however, these viruses are associated with chronic conditions, including cancer, due to their ability to establish lifelong latent infection in B-cells. In immunosuppressed individuals, yHVs pose a high risk of viral reemergence from latency that can lead to cancer. Murine gammaherpesvirus 68 (MHV68) is homologous to EBV and is an animal model used to study yHV-driven lymphomagenesis. yHV-associated lymphomas consist of infected cells; they likely exhibit virus-specific vulnerabilities and resistance mechanisms. To systematically investigate these vulnerabilities, we developed a robust preclinical B-cell lymphoma model using two lymphoma models, the A20 and the Eμ-myc (EM) B-cell model. Initial infection studies using a LANA-β-lactamase fusion reporter virus showed that both B-cell lines exhibit low infection efficiency. Therefore, to investigate viral vulnerabilities, we generated virus-matched cell lines for each B-cell lymphoma model by using a drug-selectable GFP-tagged MHV68 reporter virus to select and propagate the rare (virus+) B cells. We then validated the virus(+/-) status of these matched cell lines and defined baseline GFP fluorescence expressions. Furthermore, (virus+) cell lines respond to common chemical inducers of viral reactivation, which increase viral gene expression and DNA replication. The successful generation of these drug-selectable virus-matched cell lines provides a robust platform for studying yHV-driven lymphomagenesis. By enabling this direct comparison of (virus-) versus (virus+) lymphomas, our model will facilitate the development of therapeutic approaches targeting viral vulnerabilities in yHV-associated lymphomas.

Characterizing Staphylococcus aureus oxoproline metabolism and role in skin infection

Sydney Hall

Poster # 9

The bacterial pathogen Staphylococcus aureus is the global leading cause of skin and soft tissue infections and is a frequent colonizer of the epidermis. Surprisingly, ~25% of the free amino acid content of the human epidermis is 5-oxoproline (OP), a nonstandard, modified form of proline. OP metabolism has only been characterized in a handful of prokaryotes and has not been documented in any species of the Staphylococcus genus or skin-commensal bacterium to date. We have discovered an operon in S. aureus that contains genes that are homologous to an oxoprolinase enzyme and transporter. RNA-seq revealed that growth in both in vitro and in vivo skin conditions upregulates this locus, and we used mass spectrometry to show that OP is depleted over time from the media of growing cultures. Deletion and transposon mutants in these genes grow poorly in a chemically-defined minimal media supplemented with OP compared to wildtype, and this phenotype is complementable. Preliminary in vitro data suggests OP metabolism also increases S. aureus survival when grown with neutrophil-like cells, and transposon-sequencing implicates this locus as important during a murine abscess model. This suggests a potential role for OP metabolism in a pathogenic context, as well as a commensal-like colonization context. Overall, this is the first time a member of the Staphylococcus genus has been suggested to utilize OP, which could be important during growth on the skin and during infection. Moving forward, we will do comparative metabolomics on mutants and study the role of OP metabolism in interactions with neutrophils during infection.

Strain-dependent expression of an immunogenic adhesin-like protein that confers a colonization advantage in the gut

Luke Hanson

Poster # 10

Immunoglobulin A (IgA) targets and protects against inflammatory and pathogenic commensal microbes, including fungi, in the gut. Candida glabrata is a gut commensal that can disseminate and cause infection, but the role of IgA in regulating its colonization is unclear. Using antibiotic-treated and germ-free mouse models we identified a clinical isolate, CG27, that is highly targeted by IgA. Transcriptional analyses revealed a gene AWP11 is differentially expressed in CG27 and other IgA-targeted strains when compared to non-targeted strains. In a parallel experiment western blots and proteomic analysis were done to corroborate that IgA is targeting AWP11. We show that this protein is necessary for IgA induction by colonizing mice with an AWP11 mutant strain, generated via a CRISPR mediated frameshift mutation. These mice did not induce a specific IgA response against Awp11 or any other CG27 antigen. AWP11 is an adhesin-like wall protein and to explore its function the mutant strain was competed against CG27 in both antibiotic and non-antibiotic treated mouse models. In the antibiotic treated model CG27 demonstrated a gradual competitive advantage that is altered around a timepoint that a specific IgA response is expected, indicating a role for antibodies in altering the competitive fitness of CG27 via Awp11 binding. In the non-antibiotic competition CG27 demonstrated a much stronger competitive advantage which reveals a potential role for Awp11 in mediating a bacterial interaction that allows for C. glabrata to establish persistent colonization. Altogether these results demonstrate strain dependent variation in expression of a highly immunogenic protein that drives a colonization advantage and may reveal novel roles for IgA in regulation of C. glabrata as well as an unknown cross kingdom interaction that facilitates persistence.

Characterization of Staphylococcus epidermidis Diabetic Wound Clinical Isolates

Megan Hupka

Poster # 11

Diabetic wounds are a leading cause of amputations globally and are often infected with microbes. These wounds are classified into three classes, with class 3 being the most severe. To determine which microbes are the most prevalent, we isolated a collection of microbes from 100 patients with diabetic ulcers at the University of Colorado Anschutz. We found that the bacterium Staphylococcus epidermidis, a common colonizer of the skin, was a prevalent species in these wounds. Current knowledge on S. epidermidis leaves questions of the beneficial or detrimental nature of this bacterium, particularly in the context of the diabetic wound. Here, we characterize S. epidermidis isolates from our collection by looking at biofilm formation, antibiotic resistance, and adherence to keratinocytes. In a crystal violet assay, 81% of isolates form strong or intermediate biofilms relative to a known biofilm former. Antibiotic resistance to five common antibiotics was assessed. All isolates, aside from one, are resistant to penicillin, and 76% of isolates are also resistant to erythromycin. Conversely, 95% of isolates were susceptible to vancomycin and tetracycline. Adherence to keratinocytes varied, with an average adherence of 15%. Additionally, we utilized a murine model of diabetic wound infection to examine the impact of diabetic status on bacterial wound burden, as the role S. epidermidis plays in this context is still unknown. Isolate 409, from a class 3 clinical wound, was chosen to test in vivo alongside the lab strain 1457 since it was the only S. epidermidis isolate from a class 3 wound. From this experiment, we determined there was no significant impact of murine diabetic status on bacterial burden in the wound for 1457 and isolate 409. Future work will continue characterizing these S. epidermidis isolates through whole genome sequencing and will examine polymicrobial interactions in the context of the diabetic wound.

Interactions between Group B Streptococcus and Enterococcus faecalis in the murine diabetic wound

Alyx Job

Poster # 12

Diabetes mellitus is a chronic disease affecting more than 300 million people worldwide. Approximately 25% of people with diabetes will develop a wound in the lower limb, often a foot ulcer, at some point in their lives. These wounds are particularly susceptible to chronic infection and are the leading cause for non-traumatic lower limb amputation worldwide. Group B Streptococcus (GBS) is a Gram-positive opportunistic pathogen and is increasingly isolated in individuals with diabetic wounds. Our lab has recently collected over 400 bacterial and fungal isolates from human diabetic foot ulcers. GBS was a frequently isolated species and often co-isolated with another prevalent opportunistic pathogen, Enterococcus faecalis. Given the high co-occurrence of these species, we sought to investigate factors contributing to their interactions. We used our murine model of diabetic wound infection to evaluate the effect of GBS on E. faecalis persistence in a relevant in vivo environment. Interestingly, we observed significantly increased E. faecalis wound burdens when mice were co-infected with E. faecalis and GBS than with E. faecalis alone. We next sought to determine factors required for E. faecalis persistence in diabetic wounds using transposon sequencing (Tn-seq). Using the arrayed E. faecalis OG1RF SMarT library and GBS strain CJB111, we inoculated the wounds of diabetic mice. Tissues were harvested and plated on selective media 24 hours post-infection. Extracted gDNA was sent for next-generation sequencing at the University of Minnesota Genomics Center. Using TRANSIT2 analysis, we compared the transposon abundance from tissues infected with E. faecalis alone vs E. faecalis and GBS to determine how GBS influences E. faecalis gene requirement in the murine diabetic wound. Results indicate that multiple cell wall factors may be important for GBS interactions. Future work will investigate the role of enterococcal pili, EPA, and xpaC/telA in the diabetic wound environment.

Meningeal cell contribution to barrier breakdown during neonatal bacterial meningitis

Sophia Kim

Poster # 13

Due to incomplete maturation of physiological barriers, newborns are highly susceptible to infections caused by invading pathogens such as bacteria. Once in the central nervous system, bacteria can induce local inflammation of the meninges, causing meningitis. Located between the dura and inner arachnoid mater, the meningeal arachnoid barrier (AB) functions as a size-restrictive barrier between the CSF and the periphery. This barrier property is regulated by intercellular junctional proteins and our previous study showed that the absence of AB results in a significantly higher level of Group B Streptococcus (GBS) bacteria, the primary cause of neonatal bacterial meningitis, in the meninges. However, how the infection alters cellular and functional properties of the barrier, as well as the local inflammatory response has never been investigated.Using a GBS-induced bacterial meningitis model in neonatal mice, we found that the AB became significantly more permeable, which coincided with disruption of Claudin-11+ tight junction organization and elevated meningeal expression of proinflammatory molecules IL-6, TNF-α and CXCL1. Histological data showed that the border-associated macrophages (BAMs), the major resident immune cells of the meninges, undergo structural and molecular changes in infected mice. To investigate whether local immune response from BAMs contributes to barrier disruption, we pharmacologically depleted BAMs prior to inducing meningitis. However, the AB permeability was not affected and there was limited change in the production of proinflammatory molecules. Together, these results suggest that the disruption of AB during acute bacterial infection in neonates likely involves other inflammatory-responsive cells such as neutrophils, fibroblasts or endothelial cells or the bacteria itself. Ongoing studies focus on discovering the major driver of proinflammatory response and the mechanism underlying Claudin-11 disruption upon infection.

Divergent outcomes of MHV68 infection in myeloid cells implicate cytokine signaling in lytic restriction

Rachael Kostelecky

Poster # 14

The gammaherpesviruses (gHVs) are viruses that establish lifelong infection and are associated with multiple types of cancer and chronic inflammation. Immunosuppression is one major risk factor for the development of virus-associated disease, yet how the immune system controls infection remains unclear. To study this, our lab uses murine gammaherpesvirus 68 (MHV68), a small animal model that is genetically and biologically related to the human gHVs. While these viruses infect multiple cell types, how virus infection is regulated in macrophages, an early target of infection, is poorly understood. Using in vitro and in vivo models, our lab has shown that macrophages are readily infected by MHV68, but only a small fraction of cells undergo active virus replication. Single-cell RNA sequencing further revealed that cytokine signaling may limit virus replication in macrophages. To investigate this, we used cytokine treatments and pharmacological inhibitors to probe the potential role of JAK/STAT signaling in controlling virus replication. Canonical cytokine signaling via the JAK/STAT pathways activates different STAT transcription factors that bind to gene promoters to regulate transcription. While interleukin-4 (IL-4) induces STAT6 to promote virus replication, type I and II interferons induce STAT1 to trigger an antiviral response. Our data demonstrates that interferon-induced STAT1 potently suppresses the ability of IL-4 to promote virus replication in macrophages. Additionally, we found that inhibition of JAK/STAT signaling with Ruxolitinib (RUX) prominently increased the frequency of cells initiating virus replication, suggesting that basal JAK/STAT signaling may be a major barrier to virus replication in macrophages. Our data further identified that many of these effects may be mediated by influencing expression of the viral replication and transcription activator (RTA) gene, the major viral gene that initiates virus replication. RUX strongly induced RTA expression

Mechanisms of Staphylococcus aureus resistance to commensal antimicrobial peptides

Anna-Sophia Leon

Poster # 15

Staphylococcus aureus is an opportunistic pathogen that can transiently colonize the skin. The skin is a polymicrobial environment in which skin commensals can produce antimicrobial peptides (AMPs) for niche competition. Of the coagulase-negative staphylococci (CoNS) skin commensals, S. epidermidis is the most abundant species and often co-isolated with S. aureus. S. epidermidis can secrete AMPs, such as epidermin, which exhibits broad antimicrobial activity against skin commensals. Knowledge of S. aureus resistance mechanisms to epidermin are not well understood and some reports suggest it is naturally resistant. We hypothesized to observe strain level variation in S. aureus resistance to epidermin and that a two-component regulatory system (TCS) may impact resistance. To start, 23 divergent S. aureus strains were tested for susceptibility to epidermin using spot assays with live bacteria and cell-free conditioned media, and 14/23 strains were susceptible. We then took a genetic approach that revealed a non-significant trend for S. aureus strains that harbored the type II vSAb genomic island to be less susceptible to epidermin, compared to other forms of the island. The type II vSAb genomic island encodes a bacteriocin of S. aureus (Bsa) which is an AMP similar to epidermin, and we speculate that the Bsa immunity genes may play a role in epidermin protection. To investigate S. aureus regulation of epidermin resistance, we tested the S. aureus strain LAC and 15 TCS mutants against epidermin using a spot assay. We found that the less investigated NsaRS TCS exhibited significantly greater susceptibility compared to WT, suggesting a novel role for NsaRS in epidermin resistance. This raises the possibility that NsaRS is critical for S. aureus protection from commensal AMPs. Altogether, these results present a new avenue of research between S. aureus and epidermin, with the goal of translationally exploring the impact of these interactions on S. aureus skin colonization.

Role of cardiolipin synthase in daptomycin resistant Enterococcus faecium

Nicole Messner

Poster # 16

Enterococcus faecium is a member of the healthy human intestinal microbiota but is also a nosocomial pathogen associated with high levels of multi-drug resistance (MDR). Due to the antimicrobial resistant nature of E. faecium, antibiotic therapy often fails and can lead to patient death. With the ongoing rise of antibiotic resistance in E. faecium, we are desperate to expand on effective treatment options. Bacteriophages (phages) are viruses that infect and kill bacteria and can be leveraged as a complementary therapeutic with antibiotics. Data from this study and others reaveals that MDR bacteria can be far more sensitive to phage and antibiotics combined compared to just one alone. We aim to further understand this in hopes that phage-antibiotic combination treatment can get utilized in a clinical setting to better combat these infections. This work focuses on the clinical MDR E. faecium strain UCH5, which is a bloodstream isolate from the University of Colorado hospital. UCH5 is resistant to many antibiotics, including the last-resort antibiotic daptomycin, with a minimum inhibitory concentration 16-times higher than the resistance threshold set by the Clinical and Laboratory Standards Institute. We have found that UCH5 can be re-sensitized to daptomycin when treated in combination with phages in vitro. Despite the promise that comes with these findings, caveats remain as mechanistic understanding behind this phenomenon is sparse and E. faecium readily evolves phage resistance. Previously it was believed that phage resistance was detrimental to the success of phage therapy, but this study isolates phage resistant mutants from the parental UCH5 strain and demonstrates that phage resistance can come with a fitness tradeoff. These isolates have growth defects and increased daptomycin sensitivity, likely due to truncations in one of the cardiolipin synthase genes (cls1), which synthesizes a major cell membrane phospholipid, cardiolipin. Variation in these proteins is

IRE1 and NOD2 Activation Confers Protection Against Salmonella Typhimurium Dissemination

Elizabeth Nail

Poster # 17

Salmonella Typhimurium (S. Typhimurium) is a gram-negative intracellular pathogen acquired through ingesting contaminated food or water and is one of the leading causes of food-acquired illnesses. Upon ingestion, S. Typhimurium invades and survives within host cells, disrupting endoplasmic reticulum (ER) homeostasis, known as ER stress. ER stress is detected by the inositol-requiring enzyme 1 (IRE1), a receptor that functions to re-establish ER balance, maintain gut epithelial barrier integrity, innate inflammatory signaling, and, if necessary, induce apoptosis. The intracellular innate immune receptors NOD1 and NOD2 (NOD1/2) also contribute to inflammatory responses downstream of IRE1 during ER stress. However, the roles of IRE1 and NOD1/2 mediated signaling in S. Typhimurium pathogenesis remain poorly understood. Here, we demonstrate that the inhibition of the kinase activity of IRE1 (by KIRA6) promotes S. Typhimurium dissemination. Additionally, IRE1 inhibition in NOD2-deficient mice further exacerbates S. Typhimurium dissemination in a sex-dependent manner. Notably, male mice deficient in both IRE1 and NOD2 signaling exhibit increased S. Typhimurium dissemination to liver and spleen and increased expression of neutrophil chemoattractant in the colon during infection. Together, our work suggests that IRE1 and NOD2 signaling protects against S. Typhimurium pathogenesis.

Analysis of in vitro and in vivo macrophage infection by murine gammaherpesvirus 68

Gracyn Nelson-Reid

Poster # 18

The gammaherpesviruses are DNA viruses that are host specific and establish lifelong infections, with the outcome of infection being highly dependent on the cell type infected. The human gammaherpesviruses are Epstein Barr Virus and Kaposi's Sarcoma Herpesvirus. Murine gammapherpesvirus 68 (MHV68) is a small animal model for gammaherpesvirus infection that allows a detailed investigation of virus-host interactions throughout the course of infection. Gammaherpesvirus infection is well-defined in certain cell types, but is less defined in myeloid cells, despite the important role of these cells in infection, dissemination and the antiviral response. We have demonstrated that MHV68 infects both J774 macrophage cells in vitro and peritoneal macrophages in vivo. With this demonstration, we can further characterize macrophage infection and cellular responses in vitro using cell lines, primary cell cultures and in vivo analysis of multiple sites of infection including lung, peritoneal cavity and spleen. Ongoing studies are investigating the impact of virus infection on macrophage function and protein expression, with the goal to compare infected and uninfected cells in the same inflammatory environment. Through these studies, we seek to understand how virus infection affects macrophage function and stability to promote lifelong infection in vivo.

Type I Interferon signaling enhances Streptococcus pneumoniae middle ear infection

Steven Shaw

Poster # 19

Streptococcus pneumoniae is a common cause of bacterial otitis media (OM), and viral co-infection is associated with increased rate of bacterial OM. However, the host factors during co-infection that facilitate increased OM remain poorly understood. A consequence of a viral infection is the induction of a type I interferon (IFN) response, which signals through the type I interferon receptor (IFNAR) to activate interferon stimulated genes. Type I IFN signaling following viral infection reduces anti-bacterial immunity in the lungs, but the impact of this signaling pathway on bacterial OM is unknown. We hypothesized that virus-induced type I IFN signaling increases S. pneumoniae invasion to the middle ear, contributing to higher OM bacterial burdens. Using a murine model, we found that intranasal treatment with the synthetic viral analog poly(I:C) was sufficient to increase S. pneumoniae invasion to the middle ear. However, poly(I:C) failed to enhance S. pneumoniae infection in IFNAR KO mice, suggesting a requirement for type I IFN signaling. Additionally, neutrophil expression of TNF-alpha was significantly lower in mice treated with poly (I:C) compared to untreated mice and IFNAR mice given poly (I:C). Increased TNF-alpha production by neutrophils correlated with improved S. pneumoniae clearance and increased neutrophil phagocytosis of S. pneumoniae. In patients with chronic OM, a similar phenotype of decreased neutrophil phagocytic capacity was observed, relative to circulating neutrophils. Ongoing studies will dissect the importance of type I interferon signaling on neutrophil-mediated clearance during viral co-infection with the goal of identifying novel immunotherapy approaches for bacterial OM.

The structural basis for coordination between tandem exonuclease resistant RNAs (xrRNAs) in dengue virus

Elizabeth Spear

Poster # 20

Flaviviruses are positive-sense single-stranded RNA viruses whose genomes contain functionally important structured RNA elements. These include exoribonuclease-resistant RNAs (xrRNAs), structures that reside at the beginning of the 3' untranslated region (UTR) where they prevent degradation of the downstream sequence by host 5’ to 3’ exoribonucleases such as Xrn1. This incomplete degradation results in accumulation of infection important non-coding subgenomic flavivirus RNAs (sfRNAs). Many flaviviruses contain two tandem xrRNAs, and multiple studies reveal that in dengue virus serotype 2 (DV2) the function of the tandem xrRNAs are mysteriously coupled. We hypothesized that there are important molecular interactions taking place between the two xrRNA structures that facilitate specific sfRNA biogenesis patterns during infection. We therefore tested the effect of mutations that altered (1) the order of the xrRNAs, (2) the spacing between them, or (3) the sequence of a short ‘single-stranded’ intervening linker, using both infections and a novel reporter system in mammalian and insect cells. These studies suggested that an A-rich linker region between the tandem xrRNAs is necessary for coupling the activities of the two xrRNAs, and therefore for determining the patterns of sfRNA formation. Also, the coupling occurs outside of authentic infection – it is an inherent feature of the RNA structure itself. Further exploration of these mutant tandem xrRNA structures with chemical probing and small angle X-ray scattering revealed that increasing the local flexibility of the A-rich linker propagates to affect the global shape of the tandem xrRNAs, and cryo-electron microscopy reveals that this linker may act to favor a specific 3-D orientation of the two xrRNAs. In summary, these studies are now the first explanation for how different structural elements in the DV2 3’ UTR can communicate and points the way to further exploration of how the global architecture of flavivirus 3’

Investigating the Role of Biofilm Formation as a Driver for Candida albicans-associated inflammation in IBD

Emma Trujillo

Poster # 21

Candida albicans is a polymorphic fungus that asymptomatically colonizes the mucosal surfaces of approximately 80% of humans, with the gut serving as its largest reservoir. In healthy individuals, this colonization is benign; however, in immunocompromised patients, C. albicans can trigger or exacerbate disease. Inflammatory Bowel Disease (IBD) encompasses chronic conditions characterized by intestinal inflammation. Notably, in human IBD patients, C. albicans has been observed to bloom in these inflamed environments. In murine models of colitis, such blooms have been associated with heightened inflammation, suggesting a potential pathogenic role. Despite this correlation, the mechanisms driving fungal proliferation and its pro-inflammatory effects remain poorly understood. To bridge this knowledge gap, we performed bulk RNA sequencing on C. albicans isolated from the gastrointestinal tracts of colitic and control mice across multiple time points. Our analysis revealed a consistent upregulation of biofilm-associated genes in colitic conditions, with BCR1, a key biofilm regulator, prominently elevated on both Days 7 and 10. Functional assays demonstrated that Bcr1 enhances fungal colonization in both inflamed and non-inflamed gut environments. In future studies, we aim to determine whether C. albicans forms biofilms in vivo as a direct response to intestinal inflammation, assess the contribution of Bcr1 to disease severity, and identify its downstream transcriptional targets during gut inflammation. Additionally, we plan to investigate host-derived factors that may drive the observed transcriptional shifts, further elucidating the complex interplay between fungal adaptation and intestinal inflammation.