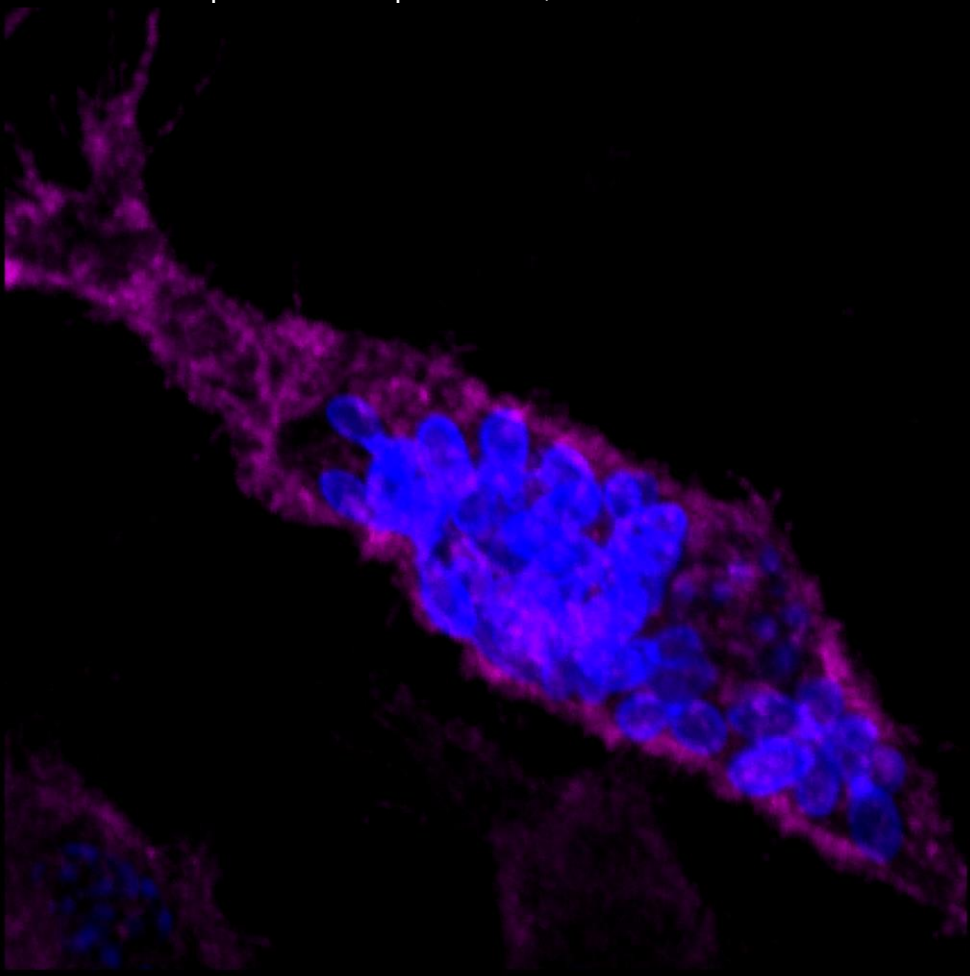
Abstracts of papers presented
at the 2025 meeting on

MICROBIAL PATHOGENESIS & HOST RESPONSE

September 8–September 12, 2025



Cold Spring Harbor Laboratory
MEETINGS & COURSES PROGRAM

Abstracts of papers presented
at the 2025 meeting on

MICROBIAL PATHOGENESIS & HOST RESPONSE

September 8–September 12, 2025

Arranged by

Melanie Hamon, *Institut Pasteur, France*
Anita Sil, *University of California, San Francisco*
Victor Torres, *St. Jude Children's Research Hospital*

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Front cover: Histoplasma is an intracellular fungal pathogen of macrophages. Confocal fluorescence microscopy image of a murine bone-marrow derived macrophage infected with wild-type *Histoplasma* yeast captured at 24 hours post-infection. Samples were stained with a fluorophore-conjugated F4/80 antibody (magenta) to detect the macrophage surface, Calcofluor White (blue) to detect *Histoplasma*, and DAPI (blue) to detect DNA. Image courtesy of Rosa Rodriguez and Anita Sil.

MICROBIAL PATHOGENESIS & HOST RESPONSE
Monday, September 8 – Friday, September 12, 2025

Monday	7:30 pm – 10:30 pm	**1** Bacterial Physiology and Phage Biology
Tuesday	9:00 am – 12:00 pm	**2** Host Immune Response to Microbes
Tuesday	2:00 pm – 5:00 pm	**3** Cell Biology of Hosts and Microbes
Tuesday	5:00 pm	*Wine & Cheese Party*
Tuesday	7:30 pm – 10:30 pm	**Poster Session I**
Wednesday	9:00 am – 12:00 pm	**4** Microbial Development and Signaling
Wednesday	2:00 pm – 5:00 pm	**5** Microbial Communities in Health and Disease
Wednesday	7:30 pm – 10:30 pm	**Poster Session II**
Thursday	9:00 am – 12:00 pm	**6** Molecular Genetics of Host-Pathogen Interactions
Thursday	1:30 pm – 4:00 pm	**Poster Session III**
Thursday	4:00 pm – 5:00 pm	**Keynote Speaker**
Thursday	6:00 pm – 7:00 pm 7:00 pm	*Concert* *Cocktails and Banquet*
Friday		*Departure*

All times shown are US Eastern: Time Zone Converter

Mealtimes at Blackford Hall are as follows:
Breakfast 7:30 am-9:00 am
Lunch 11:30 am-1:30 pm
Dinner 5:30 pm-7:00 pm

Bar is open from 5:00 pm until late

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PROGRAM

MONDAY, September 8—7:30 PM

SESSION 1 BACTERIAL PHYSIOLOGY AND PHAGE BIOLOGY

Chairperson: **Joe Bondy-Denomy,** University of California, San Francisco

Bacteriophage defense and anti-defense in *Pseudomonas aeruginosa*
Joseph Bondy-Denomy.
Presenter affiliation: University of California, San Francisco, San Francisco, California. 1

A dual functioning prophage accessory protein drives MRSA pathogenesis
Adam M. Pickrum, Amelia Pi, Victor J. Torres.
Presenter affiliation: St. Jude Children's Research Hospital, Memphis, Tennessee. 2

Inducible transposon mutagenesis identifies bacterial fitness determinants during murine infection
Ian W. Campbell, David W. Basta, Emily J. Sullivan, Julia A. Hotinger, Karthik Hullahalli, Mehek Garg, Matthew K. Waldor.
Presenter affiliation: Brigham and Women's Hospital, Boston, Massachusetts; Harvard Medical School, Boston, Massachusetts. 3

The role of a conserved orf in metal homeostasis in Gram positive pathogens
Nicholas Faiola, Reid Wilkening, Abbey Behler, Lindsey Burcham, Laura Cook.
Presenter affiliation: Binghamton University, Binghamton, New York. 4

Evolution of immunity across domains of life
Aude Bernheim.
Presenter affiliation: Institut Pasteur, Paris, France. 5

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TUESDAY, September 9—9:00 AM

SESSION 2 HOST IMMUNE RESPONSE TO MICROBES

Chairperson: **Denise Monack,** Stanford University School of Medicine, California

TUESDAY, September 9—2:00 PM

SESSION 3 CELL BIOLOGY OF HOSTS AND MICROBES

Chairperson: **Suzanna Salcedo,** University of Wisconsin, Madison

TUESDAY, September 9—5:00 PM

Wine and Cheese Party

TUESDAY, September 9—7:30 PM

POSTER SESSION I

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WEDNESDAY, September 10—9:00 AM

SESSION 4 MICROBIAL DEVELOPMENT AND SIGNALING

Chairperson: **Michael Lorenz,** University of Texas McGovern Medical School, Houston

Antibiotics accumulate to therapeutic levels in *Klebsiella pneumoniae* liver abscesses but fail to eradicate antibiotic tolerant populations
Michelle Angeles-Solano, Zajeba Tabashsum, Jamie D. Liu, Kimberly A. Walker, Sarah E. Rowe.
Presenter affiliation: University of North Carolina, Chapel Hill, North Carolina. 27

Monitoring bacterial heme transfer *in vitro*—A FlAsH-y new approach
Corbett C. Ouellette, Joanna A. Quaye, Giovanni Gadda, Zehava Eichenbaum.
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Roles of the MAP kinase SakA in stress responses and virulence of *Aspergillus fumigatus*
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Vanessa Sperandio.
Presenter affiliation: Vanessa Sperandio, Madison, Wisconsin. 30

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SESSION 5 MICROBIAL COMMUNITIES IN HEALTH AND DISEASE

Chairperson: **Joseph Zackular,** Perelman School of Medicine, University of Pennsylvania, Philadelphia

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Presenter affiliation: University of Colorado Anschutz Medical Campus, Aurora, Colorado. 31

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Presenter affiliation: University of Pennsylvania, Philadelphia, Pennsylvania; Children's Hospital of Philadelphia, Philadelphia, Pennsylvania. 42

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Presenter affiliation: NYU Grossman School of Medicine, New York, New York. 44

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Michael Fischbach.
Presenter affiliation: Stanford University, Stanford, California.

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KEYNOTE SPEAKER

Sarah Gaffen
University of Pittsburgh

"Seventeen forever—IL-17 signaling and immunity to *Candida albicans"*

THURSDAY, September 11—6:00 PM

CONCERT
Grace Auditorium

Sergey Antonov and **Ilya Kazantsev**
Cello / Piano duo

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COCKTAILS and BANQUET

FRIDAY, September 12

Departure

POSTER SESSION I

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Presenter affiliation: Texas A&M College of Medicine, Bryan, Texas; Vanderbilt University Medical Center, Division of Infectious Diseases, Tennessee. 144

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Presenter affiliation: University of California San Diego, La Jolla, California. 205

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Presenter affiliation: Tufts University School of Medicine, Boston, Massachusetts; Tufts University Graduate School of Biomedical Sciences, Boston, Massachusetts. 209

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BACTERIOPHAGE DEFENSE AND ANTI-DEFENSE IN PSEUDOMONAS AERUGINOSA

Joseph Bondy-Denomy

University of California, San Francisco, Microbiology & Immunology, San Francisco, CA

Pseudomonas aeruginosa is a common human pathogen with severe antibiotic resistance in the clinic. In addition to multiple genes that confer antibiotic resistance, this organism is chalked full of phage defense mechanisms that act inside the cell (i.e. Gabija, CBASS, Thoeris, CRISPR-Cas, Jumbo phage killer, and Restriction-modification) as well as those that act on the outside (O-antigen variability, exopolysaccharide production, receptor masking, distinct pilin subtypes, and pilus glycosylation). Whether a phage can infect and lyse a given strain is generally impossible to predict or explain, but nothing could be more important for guiding phage therapeutic outcomes. Here, I will discuss our latest efforts towards uncovering the mechanisms of phage exclusion that operate in model lab and clinical strains of P. aeruginosa, and the cognate phage counter-response. Through our work, we hope to of holistically understand and predict phage outcomes.

A DUAL FUNCTIONING PROPHAGE ACCESSORY PROTEIN DRIVES MRSA PATHOGENESIS

Adam M Pickrum, Amelia Pi, Victor J Torres

St. Jude Children's Research Hospital, Host-Microbe Interactions, Memphis, TN

Staphylococcus aureus is a highly successful human pathogen and the emergence of community-associated methicillin resistant *S. aureus* (CA-MRSA) lineage USA300 at the turn of the 21st century represented a major evolutionary shift. Unlike hospital-associated lineages, USA300 can infect otherwise healthy individuals, though the genetic traits that have allowed the continued dominance of USA300 in the community are still unclear. A hallmark in the evolution of USA300 is the acquisition of the Sa2 prophage, which carries the well-studied Panton Valentine Leukocidin (PVL), a cytolytic toxin linked epidemiologically with invasive *S. aureus* infections. Using primary human neutrophils, which serve as critical first-line defenders against infection, we found that *S. aureus* harbors an intracellular lifestyle in these professional phagocytes when using low bacterial burdens, mimicking the initial breach. This phenotype was independent of any major cytolysin, including PVL. Indeed, mutations are known to accumulate in classical virulence loci of USA300 hospital-associated isolates, generating low cytotoxic potential *in vitro* and *in vivo*. We therefore posit that the USA300 lineage encodes additional factors that protect against neutrophil-mediated killing to promote disease. A transposon mutagenesis screen was used to identify *S. aureus* mutants with increased susceptibility to killing by human neutrophils. Among the most impaired mutants in our screen was an insertion into a Sa2 prophage gene that encodes for a small protein involved in the bacteriophage lytic cycle. Here, we have uncovered a novel function of this prophage accessory protein which is to protect *S. aureus* in stressful conditions. Consistent with this function, mutation of this prophage element attenuates *S. aureus* virulence *in vivo* during pneumonia and bloodstream infections. Thus, we describe a novel mechanism by which *S. aureus* USA300 repurposes an epidemiologically important prophage to protect against neutrophil attack through a bifunctional peptide.

INDUCIBLE TRANSPOSON MUTAGENESIS IDENTIFIES BACTERIAL FITNESS DETERMINANTS DURING MURINE INFECTION.

Ian W Campbell[1,2], David W Basta[3], Emily J Sullivan[1,2], Julia A Hotinger[1,2], Karthik Hullahalli[1,2], Mehek Garg[1,2], Matthew K Waldor[1,2,4]

[1]Brigham and Women's Hospital, Division of Infectious Diseases, Boston, MA, [2]Harvard Medical School, Department of Microbiology, Boston, MA, [3]Brigham and Women's Hospital, Harvard Medical School, Department of Pathology, Boston, MA, [4]Hughes Medical Institute, HHMI, Boston, MA

*Ian W. Campbell and David W. Basta contributed equally to this work.

Transposon insertion sequencing (a.k.a., Tn-seq, TraDIS, INSeq) is a powerful tool for genome-scale functional genetics in bacteria. However, implementing Tn-seq in natural microbial environments, like animal models of infection, is limited by population bottlenecks, which can confound analysis by causing a stochastic loss of mutant diversity. We developed 'InducTn-seq' to overcome this limitation by inducing mini-Tn5 transposition after host-imposed bottlenecks. Applying InducTn-seq to *Citrobacter rodentium* in a mouse model of infectious colitis bypassed a highly restrictive host bottleneck, generating a diverse population of over 500,000 unique transposon mutants compared to ~100 recovered by traditional Tn-seq. This *in vivo* screen quantified the impact of thousands of genes on bacterial fitness within the intestine, including the previously identified virulence island and metabolic pathways previously uncharacterized during infection. Unexpectedly, InducTn-seq discovered that the *C. rodentium* type I-E CRISPR-Cas locus is required for colonization. Mechanistic studies revealed that a cryptic toxin encoded within the CRISPR-Cas locus is conditionally active during infection unless it is repressed by the CRISPR-associated targeting complex Cascade, functioning as a toxin-antitoxin system to addict cells to CRISPR activity within the host. Furthermore, a screen for regulators of *C. rodentium* CRISPR-Cas activity found that the oxygen-responsive transcription factor Fnr activates CRISPR-Cas immunity within the anoxic mouse intestine, resulting in enteric-specific activity. Since Fnr-dependent regulation is predicted in ~41% of Enterobacteriaceae *cas3* orthologs, we propose that anoxic regulation of CRISPR-Cas immunity is an adaptation that protects Enterobacteriaceae against intestinal predation. Moreover, our findings highlight the potential of InducTn-seq for genome-scale forward genetic screens in bacteria.

THE ROLE OF A CONSERVED ORF IN METAL HOMEOSTASIS IN GRAM POSITIVE PATHOGENS.

Nicholas Faiola[1], Reid Wilkening[2], Abbey Behler[3], Lindsey Burcham[3], Laura Cook[1]

[1]Binghamton University, Biological Sciences, Binghamton, NY, [2]University of Colorado - Anschutz, Pediatrics, Denver, CO, [3]University of Tennessee - Knoxville, Microbiology, Knoxville, TN

In bacteria, zinc homeostasis is tightly regulated and is especially important for pathogenic species to survive and evade immune defenses. During infection, local zinc levels can be low, as the host sequesters zinc and other essential metals away from pathogens using proteins such as calprotectin and calgranulin C, termed nutritional immunity. These host defenses have pushed bacteria to evolve various mechanisms to overcome limited metal supply during infection. In this study, we examine a putative sRNA/peptide pair, Srn151/SAK_RS00910 in Group B Streptococcus (GBS) as well as it's conservation in other related species of the Bacilli class. We provide evidence that this locus is upregulated under metal deplete conditions and that the ORF is translated into a peptide in GBS. Additionally, we inspect amino acid conservation of RsaX20 domain proteins and genomic neighborhoods of this gene in GBS, Group A Streptococcus (GAS), *S. aureus*, and *E. faecalis* as well as examine expression levels in these species under zinc limiting conditions. Lastly, we present data indicating that a knockout of the Srn151 locus in GBS results in colonization deficiencies in a murine vaginal colonization model. Understanding the role of this conserved peptide may offer new insights into bacterial metal regulation and identify potential targets for antimicrobial strategies aimed at disrupting essential metal ion balance in pathogenic species.

EVOLUTION OF IMMUNITY ACROSS DOMAINS OF LIFE

Aude Bernheim

Institut Pasteur, Genomes and Genetics, Paris, France

Immune defence mechanisms exist across the tree of life in such a wide diversity that the immune mechanisms of bacteria (antiphage systems) were considered unrelated to immunity of eukaryotes. However, recent discoveries unveiled hundreds of novel antiphage systems. Among this diversity of novel bacterial immune mechanisms, it emerged that a subset of antiphage defense systems are conserved in eukaryotes and are major actors of diverse immune pathways, leading us to revisit this paradigm and propose the concept of ancestral immunity as protein domains used in prokaryotic and eukaryotic immunity. I will discuss the evolutionnary dynamics of immunity across domains of life and how existence of ancestral immunity can lead to discoveries in prokaryotes and eukaryotes.

EPIGENETIC CONTROL OF CELL FATE: DNA METHYLATION REGULATES *SPOIIE* EXPRESSION TO PROMOTE SPORULATION AND DEVELOPMENTAL FLEXIBILITY IN *CLOSTRIDIOIDES DIFFICILE*

Pola Kuhn, John W Ribis, Shailab Shrestha, Aimee Shen

Tufts University School of Medicine, Department of Molecular Biology and Microbiology, Boston, MA

Methylation of the bacterial genome is a widespread phenomenon and can regulate gene expression, though the mechanisms underlying epigenetic regulation are often poorly understood. In *Clostridioides difficile*, the orphan methyltransferase CamA promotes sporulation, a process critical for persistence and transmission of this nosocomial pathogen. While CamA was previously shown to enhance activation of the sporulation-specific sigma factor, σ^F, its direct regulatory targets were unknown. Here, we show that methylation of a single CamA motif upstream of the sporulation gene *spoIIE* is sufficient to promote its transcription, increasing the frequency of σ^F activation and enhancing sporulation efficiency. Surprisingly, the CamA-dependent increase in *spoIIE* expression also drives premature activation of σ^F in predivisional cells in over a third of the sporulating population. While σ^F activation in the forespore irreversibly commits a cell to completing sporulation, cells that activate σ^F in the predivisional cell can abort the sporulation program and resume vegetative growth. Thus, CamA enhances sporulation without compromising a population's capacity to adapt to fluctuating environmental conditions. Finally, we find that CamA provides a significant fitness advantage during infection that is largely independent of its role in sporulation, suggesting it regulates additional genes that confer important functions during infection. These findings highlight CamA, which is highly specific to *C. difficile*, as a multifaceted regulator of bacterial fitness during infection and, therefore, a promising antimicrobial target.

IN SITU ARCHITECTURE OF THE ENDOSYMBIONT-HOST INTERFACE

Carrie L Shaffer[1,2,3]

[1]University of Kentucky, Veterinary Science, Lexington, KY, [2]University of Kentucky, Markey Cancer Center, Lexington, KY, [3]University of Kentucky, Microbiology, Immunology, and Molecular Genetics, Lexington, KY

A paradigm for understanding mutualistic adaptations underlying microbial endosymbiosis, maternally-transmitted *Wolbachia* is the most prevalent bacterial infection in the animal kingdom. Numerous experimental challenges presented by the *Wolbachia* obligate intracellular lifestyle have impeded the discovery of bacterial mechanisms governing host manipulation and germline invasion. To gain comprehensive insight into structural attributes underpinning the endosymbiont-host interface, here we used cryo-electron tomography to image isolated *Wolbachia* cells in the native state at three-dimensional, nanometer-scale resolution. Our studies identified multiple novel *Wolbachia* cellular features including membranous protrusions, periplasmic macromolecular complexes, cytoplasmic-localized cluster densities, and ladder-like filamentous structures associated with the bacterial cell surface. We speculate that analogous to rodlet filaments assembled on the *Streptomyces* spore coat, the *Wolbachia* ladder-like structure may serve as a specialized motility mechanism that enables bacterial translocation to specific host cell compartments during embryogenesis and somatic tissue dissemination. In addition, we present the first *in situ* structure of the specialized α-proteobacterial type IV secretion system (T4SS) apparatus. We provide structural evidence that the wMel T4SS nanomachine exhibits architectural similarities to the pED208 F-pilus system including the biogenesis of conjugative pili that polymerize on the cell surface and extend several hundred nanometers. Coupled with integrative structural modeling, we show that in contrast to other proteobacterial T4SS machineries, the wMel T4SS outer membrane complex assembles an electron-dense base plate structure in the periplasm predicted to comprise VirB9 oligomers complexed with cognate VirB10 subunits that form extended antennae projections surrounding the translocation channel pore. Collectively, our *in vivo* visualization studies provide an unprecedented view into enigmatic *Wolbachia* cell biology and highlight unique structural adaptations that reinforce evolutionarily ancient host-microbe interactions.

MICROBIAL ANTAGONISM VIA TRANSLATION-DEPENDENT mRNA DOWNREGULATION

Nicole Marino[1,2], Milan Gerovac[3], Matthew Johnson[2], Anya Flood Taylor[1], Theresa Astmann[1], Heloise Carion[2], Kristi Zoga[1], Surabhi Haniyur[2], Jorge Rodriguez[1], Joseph Bondy-Denomy[2]

[1]University of Pennsylvania, Department of Pathobiology, Philadelphia, PA, [2]University of California, San Francisco, Department of Microbiology and Immunology, San Francisco, CA, [3]Helmholtz Centre for Infection Research, Complexes in Phage-Infected Cells, Braunschweig, Germany

Bacterial CRISPR-Cas systems cleave the genomic DNA of bacteriophage and other mobile genetic elements. Many temperate phages encode anti-CRISPR (Acr) proteins to inhibit CRISPR-Cas and stabilize lysogeny. We previously reported that a Moraxella bovoculi prophage encodes an anti-CRISPR, AcrVA2, that inhibits Cas12a. Here, we show that AcrVA2 unexpectedly inhibits Cas12a biogenesis by binding to the nascent Cas12a polypeptide and triggering degradation of its mRNA. Mutations in the first fifteen amino acids of Cas12a abolish binding, downregulation, and inhibition by AcrVA2, while altering the Cas12a codon sequence and promoter has no effect. This novel mechanism of microbial antagonism enables AcrVA2 to recognize conserved residues in Cas12a and trigger its destruction before it is fully expressed. The broad distribution of this protein family across diverse bacterial classes and species, including where Cas12a systems are not found, suggests that it may use the same mechanism against other proteins.

EOSINOPHILS ENHANCE GRANULOMA-MEDIATED CONTROL OF PERSISTENT SALMONELLA INFECTION

Daniel Butler[1], Blanda Di Luccia[1], José Vilches-Moure[2], Denise Monack[1]

[1]Stanford University School of Medicine, Microbiology and Immunology, Stanford, CA, [2]Stanford University, Comparative Medicine, Stanford, CA

Salmonella enterica can persist asymptomatically within tissues for extended periods. This remarkable feat is achieved through intricate host-pathogen interactions in immune cell aggregates called granulomas, wherein Salmonella find favorable cellular niches to exploit while the host limits its expansion and tissue dissemination. Here, using a mouse model of persistent Salmonella infection, we identify a host-protective role of eosinophils in control of Salmonella Typhimurium (STm) infection within the mesenteric lymph nodes (MLN), the main lymphoid tissue of STm persistence. Combining spatial transcriptomics and experimental manipulations, we found that macrophages responding to STm infection recruited eosinophils in a C-C motif chemokine ligand 11 (CCL11)-dependent manner and enhanced their activation. Eosinophil deficiencies increased Salmonella burdens, which was associated with altered granuloma size and impaired type-1 immunity in the MLN. Thus, eosinophils play a vital role in restraining Salmonella exploitation of granuloma macrophages at a key site of bacterial persistence.

INTERCELLULAR CROSSTALK IN HOST DEFENSE AGAINST ASPERGILLUS

Tobias M Hohl[1,2]

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Aspergillus fumigatus is an opportunistic mold pathogen that humans inhale on a daily basis and clear in a silent and asymptomatic manner. In patients with qualitative or quantitative defects in myeloid cell function, the infectious propagules, termed conidia, can germinate into filamentous hyphae in the lung and cause life-threatening invasive aspergillosis. The central nervous system is the most common site of extrapulmonary dissemination.

Effective host defense depends local licensing of myeloid phagyctes to achieve their full spectrum of antifungal properties at the site of infection. In this talk, I will outline intercellular circuits that couple fungal recognition to in situ activation of neutrophils and the induction of NADPH oxidase, a critical effector system. First, I will discuss the early and critical role of GM-CSF in host defense in the lung and central nervous system, and outline tissue-specific differences in GM-CSF cellular sources. Second, I will discuss the role of lung-infiltrating plasmacytoid dendritic cells (pDCs) in pulmonary host defense and recent advances in understanding how pDC-derived interferons activate the neutrophil oxidative burst by controlling the expression of a single NADPH oxidase subunit. Collectively, this work will introduce a novel model to study aspergillosis in the central nervous system and highlight GM-CSF and pDC-dependent circuits that enhance host defense in an organ-specific manner.

EMERGENCE OF HOST AAA ATPASE, VCP/P97, AS A CYTOSOLIC IMMUNE EFFECTOR AGAINST INTRACELLULAR PATHOGENS

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Successful elimination of infectious microbes requires an efficient surveillance system, enabling quick pathogen sensing and clearance. Apart from the classical phago-lysosomal pathways, for detection and elimination of pathogens, ubiquitin-proteasomal system is known to be pivotal. Along with lipopolysaccharide, Degron motifs present in surface proteins of phylogenetically diverse pathogens are recognized and decorated with K48-polyubiquitin chains by host ubiquitination machinery. This directs the pathogens for clearance by the host proteasomal system. However, significantly smaller size of the proteasomes compared to bacteria casts doubt on this paradigm. We unveiled a novel mechanism for clearance of these ubiquitinated pathogens involving a host AAA ATPase, VCP/p97, which using its chemo-mechanical force-based function, extracts the ubiquitinated proteins from bacterial membrane. Extractions of these surface proteins cause extensive membrane rupture, triggering leaching of bacterial internal contents and subsequent death. This distinct innate antimicrobial function of p97 protects the host against variety of lethal bacterial infections.

SUPPRESSION OF EXTRACELLULAR VESICLES BY *YERSINIA PESTIS*: ANOTHER MECHANISM TO INHIBIT INFLAMMATION

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Extracellular vesicles (EVs) are produced by all cells and mediate intercellular communication. Interestingly, the cargo (proteins, lipids, and small RNAs) packaged within EVs can change based on the physiological state of the cell. In the case of myeloid cells, interactions with pathogens can both increase the production of EVs and the incorporation of cargo to promote inflammation. Despite their importance in cellular communication, the role of EVs during the host response to bacterial infections remains poorly defined. *Yersinia pestis* actively modulates the responses of macrophages and neutrophils by translocating Yop effector proteins directly into cells via a type three secretion system. In addition to inhibiting the production of inflammatory lipids and proteins, the Yops also dysregulate signaling pathways necessary for endocytosis (phagocytosis) and exocytosis (degranulation). Because of this disruption of vesicular trafficking, it is likely that *Y. pestis* also inhibits EV biogenesis by myeloid cells. Using the pneumonic plague model, we discovered stark changes in EV biogenesis over the course of infection. Moreover, we showed that EVs produced during the early stages of disease lack the same proinflammatory potential as those isolated later during infection. Using primary human neutrophils, we also identified the Yop effectors responsible for disruption of EV biogenesis, Yop-specific changes in EV cargo, and the impact of Yop-mediated disruption of EVs on inflammation, immune cell activation, and antimicrobial functions. Together, these data demonstrate for the first time that *Y. pestis* manipulates EV biogenesis in ways that are likely to contribute to the pathogen's ability to quickly suppress inflammation in order to colonize the host.

UNDERSTANDING THE IMPACT OF SEX ON IMMUNITY IN THE BLADDER

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Our group is interested in understanding how biological sex influences mucosal immunity. To study this, we use mouse models of bladder diseases and human cohorts. Urinary tract infections are profoundly sex-biased, impacting nearly 50% of all women but only 5-10% of men. Additionally, while both sexes are at significant risk of reinfection, women are more likely to have recurrent infection, whereas men develop chronic UTI. Our recent work demonstrates that resident macrophages impair the adaptive response, and that IL-17 is a critical player in resolution of infection. We also identified events leading to the development of antigen-specific tissue-resident T cells in the bladder that are necessary and sufficient for protection against recurrent infection. We have identified innate and adaptive immune responses that are divergent between the sexes and are now determining how these responses can be immunomodulated for improved therapeutics that obviate the need for antibiotics to treat multidrug resistant uropathogens.

THE DANRI REGULATORY SYSTEM IN UROPATHOGENIC *ESCHERICHIA COLI* SUBVERTS NEUTROPHIL RESPONSES

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Uropathogenic *Escherichia coli* (UPEC), the leading cause of urinary tract infections (UTI) worldwide, must adapt to the hostile environment of the bladder to successfully establish infection. Within the bladder, UPEC confronts the host immune system, particularly infiltrating neutrophils and their antimicrobial neutrophil extracellular traps (NETs). Our study identifies a novel two-gene regulatory system in UPEC, termed DanRI (Defense against neutrophil Regulator and Inhibitor), which is induced in response to NETs. This system is present in 29% of UPEC isolates and is located within the UPEC pathogenicity island $PAI_{UTI89}II$. DanRI modulates key virulence traits, including flagellar biosynthesis and stress response pathways, through a mechanism in which the inhibitor DanI suppresses the function of the transcriptional regulator DanR. Through this regulatory activity of DanRI, UPEC attenuates neutrophil reactive oxygen species production and inhibits NET formation. Importantly, DanRI is essential for UPEC fitness and persistence in a murine model of UTI. Collectively, these findings establish DanRI as a previously uncharacterized regulatory system that enables UPEC to evade neutrophil-mediated defenses and promote pathogenesis.

THE EMERGING FUNGAL PATHOGEN *CANDIDA AURIS* INDUCES IFNγ TO COLONIZE MAMMALIAN HAIR FOLLICLES

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Public health alarm concerning the emerging fungus *Candida auris* is fueled by its antifungal drug resistance and propensity to cause deadly outbreaks. Persistent skin colonization drives transmission and lethal sepsis although its basis remains mysterious. We compared the skin colonization dynamics of *C. auris* with its relative *C. albicans*, quantifying skin fungal persistence and distribution and immune composition and positioning. *C. auris* displayed a higher propensity to colonize hair follicles and avidly bound to human hair. While *C. albicans* triggered an effective sterilizing type 3/17 antifungal immune response driven by IL-17A/F-producing lymphocytes, *C. auris* triggered a type 1, IFNγ-driven immune response targeting hair follicles. Rather than promoting fungal clearance, IFNγ enhanced *C. auris* skin colonization by acting directly on keratinocytes impairing epithelial barrier integrity and repressing antifungal defense programs. *C. auris* exploits focal skin immune responses to create a niche for persistence in hair follicles.

DECIPHERING THE ROLE OF BACTERIAL SECRETED EFFECTORS: WHY IS BRUCELLA GOING NUCLEAR?

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The nucleus is widely targeted by bacteria for modulating cellular defense mechanisms and enhancing host colonization. We have recently discovered that this is also the case for *Brucella*, a highly pathogenic bacterium that causes one of the most prevalent zoonotic diseases worldwide. One of the key features of its virulence is its ability to extensively replicate intracellularly by injecting bacterial proteins into host cells, thereby promoting infection. We have shown that two new effectors, NyxA and NyxB, modulate the spatial dynamics of host nucleus proteins during infection. Yet their contribution to disease pathology remains unknown. We have now found that NyxA and NyxB are actively imported into the nucleus by specific importins, where they anchor to host chromatin in the vicinity of PML-nuclear bodies. We will discuss how this enables the Nyx effectors to control the expression of specific host proteins essential for cell-to-cell junction integrity, placing the Nyx effectors at the center of the pathology of the reproductive system caused by *Brucella* infection.

NEUTROPHIL EXTRACELLULAR TRAPS (NETs) ENHANCE DISSEMINATION IN A CD4$^+$ T CELL-DEFICIENT MODEL OF CRYPTOCOCCOSIS

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Cryptococcus neoformans is an opportunistic fungal pathogen that disproportionately affects immunocompromised individuals, particularly those with CD4$^+$ T cell deficiencies. Since the disseminated brain infection is both the driver of mortality and the presenting illness, understanding the mechanisms of dissemination and pathogenesis in these vulnerable populations is crucial for developing effective therapies. Our goals here are to elucidate the mechanisms underlying cryptococcal dissemination and how they change in CD4$^+$ T cell-deficient mice compared to wild-type mice. CD4$^{-/-}$ mice exhibited increased susceptibility to C. neoformans infection, including higher fungal burdens in extrapulmonary organs and more rapid time-to-endpoint compared to wild-type mice. Interestingly, infected CD4$^{-/-}$ mice displayed a compensatory increase in neutrophils, both quantitatively and functionally. Elevated levels of neutrophil-associated enzymes, including elastase (measured by ELISA) and myeloperoxidase (measured by IHC), were observed in the lungs of infected CD4$^{-/-}$ mice. Furthermore, these mice showed a significant increase in the proportion of C. neoformans 'seed cells' in the lungs. This dissemination-prone cryptococcal morphotype forms in the lungs over the course of infection and exhibits enhanced ability to enter extrapulmonary organs. Seed cells are induced by phosphate. We hypothesized that neutrophil extracellular traps (NETs) are a key driver of seed cells: NETs consist of the intracellular contents of neutrophils, including DNA, extruded into the tissue environment. Indeed, NET material was sufficient to induce the formation of seed cells. These findings indicate that CD4$^+$ T cell deficiency leads to increased susceptibility to C. neoformans and the host immune system compensates with an elevated neutrophilic response. However, this compensatory mechanism appears to be detrimental, as the release of NETs by neutrophils inadvertently promotes the formation of C. neoformans seed cells, thereby enhancing fungal dissemination and exacerbating disease severity.

DIVING INTO BACTERIAL DORMANCY: EMERGENCE OF OSMOTICALLY STABLE WALL-LESS FORMS IN AN AQUATIC ENVIRONMENT

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Bacteria can respond to environmental stresses by entering a dormant state called viable but non-
culturable (VBNC) state. In this state, bacteria lose the ability to grow in routine culture media. Pathogens entering a VBNC state pose thus a significantly higher risk for human and animal health, as they are not detected by standard growth-based techniques, and can "wake up" anytime back into a culturable and virulent state. Although hundreds of species were reported to become VBNC in response to different stresses, the molecular mechanisms governing this phenotypic switch have remained largely elusive.

We have characterized the VBNC state in the Gram-positive pathogen *Listeria monocytogenes*. By combining fluorescence microscopy, cryo-electron tomography with genetic and biochemical approaches, we discovered that starvation in mineral water drives *L. monocytogenes* into a VBNC state via a unique mechanism of cell wall (CW) shedding that generates osmotically stable CW-deficient coccoid forms. Interestingly, this CW-deficient VBNC state occurs across many *Listeria* species, suggesting it may be a stress-adapting process transversal to the *Listeria* genus. Transcriptomic and gene-targeted approaches revealed the stress response regulator SigB and the autolysin NamA as major moderators of CW loss and VBNC state transition. Finally, we show the transience of this CWD dormant state, as VBNC *Listeria* revert back to a walled, vegetative and virulent state after passage in embryonated eggs. Our results reveal an original survival strategy of *Listeria* and shake the longstanding assumption of the cell envelope as a fundamental bacterial component.

RAB20 MAY CONNECT IMMUNE SIGNALING TO FUNGAL-RESTRICTIVE PHAGOSOMAL MATURATION DURING CRYPTOCOCCAL INFECTION OF MACROPHAGES.

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Cryptococcus neoformans is a fungal pathogen responsible for ~200,000 deaths yearly, mostly in immunocompromised individuals. Because it is a ubiquitous environmental yeast, infectious particles are inhaled and make their way into the lungs, encountering alveolar macrophages. There, *C. neoformans* can remain latently both extra- and intracellularly. Depending on the immune response of the host, alveolar macrophages can serve as a safe haven for replication as well as a vehicle for extrapulmonary dissemination. The mechanisms *C. neoformans* use to mediate this intracellular parasitism are not fully understood; however, this ability to survive inside phagocytes, particularly alveolar macrophages, is associated with mortality. Therefore, understanding the macrophage-cryptococcal interactions and elucidating the properties of the cryptococcal-containing phagosome (CCP) are critical steps needed to improve the management of this disease.

We have identified three populations of CCPs with different behaviors: one that gains acidification after phagocytosis but subsequently loses it; some that acidify and remain acidic; and some that never acidify. We also directly observed phagosomal membrane damage, suggesting a possible mechanism for pH manipulation. This is in contrast to phagosomes containing *S. cerevisiae*, which rapidly acidify, stay acidic, and have low levels of membrane damage. Moreover, we have identified a significant population of CCPs that display both early endosomal lipid (PI3P) and lysosomal protein (LAMP1) characteristics, a combination not normally observed. Collectively, these results suggest that *C. neoformans* can alter its phagosome and provides a potential mechanism for intracellular survival that may be driving cryptococcal pathogenesis.

Further, we have demonstrated a delay in acquisition of lysosomal markers to CCPs in comparison to controls, including the vATPase complex. When macrophages are stimulated with interferon-γ, acquisition of lysosomal markers occurs more rapidly. Host Rab20 has been shown to be interferon-γ inducible and appears to be critical for this promotion of phago-lysosomal fusion as knockdown of Rab20 delays maturation of phagosomes containing non-pathogenic cargo. Therefore, Rab20 may be linking host immune status and phagosomal properties during cryptococcal infection, playing a critical role in influencing the infection outcome. We hypothesize that *Cryptococcus* actively manipulates the phagosomal environment to avoid killing by targeting Rab GTPases and phosphoinositides, in a way that is dependent on the host's immune status.

MAPPING THE MICROBE-HOST INTERFACE IN HEALTH AND DISEASE WITH GENOMIC MICROSCOPY

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Many population-level bacterial behaviors emerge from heterogeneous, stochastic actions of individual cells. Moreover, many such behaviors are shaped by the complex, often spatial structured environments in which bacteria live. In parallel, the host response to pathogenic interactions is shaped by the massive diversity of different host cell types. Single-cell transcriptomic methods offer an exciting new window into such behaviors by providing genome-wide measures of the behaviors of individual cells. Here I will discuss our efforts to develop complementary techniques that place single-cell behaviors in space. Specifically, I will describe genomic-scale microscopy methods that can characterize the single-cell transcriptional response of both bacteria and host in the context of complex microbe-interactions that occur within the mammalian gut during health and disease. These methods allow us to directly chart how bacteria adapt to the complex micron-scale niches in the gut, and, in turn, how the host remodels both the cellular composition and spatial organization of the gut in response to pathogenic-like interactions between host and microbe. We anticipate the genomic microscopy methods we are developing in the context of microbe-host interactions in the gut may prove useful for characterizing a wide variety of questions in bacterial pathogenesis in many different tissues.

INTERPLAY OF *SHIGELLA* T3SS EFFECTORS IN INHIBITION OF EPITHELIAL CELL DEATH.

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Shigella flexneri is an intracytosolic pathogen that invades colonic epithelial cells to cause bacillary dysentery or shigellosis. *Shigella* utilizes a type III secretion system (T3SS), a membrane-embedded nanomachine to deliver effector proteins directly into the host cell cytosol. These effectors inhibit host cellular processes, including host innate immune responses, enabling *Shigella* to establish a replicative niche within intestinal epithelial cells. Invasion of *Shigella* triggers pyroptosis, an inflammatory form of cell death, via activation of caspase-4 (CASP4) inflammasome. The inflammasome processes IL-18 and gasdermin D (GSDMD). The N-terminal fragment of GSDMD proceeds to form GSDMD pores in the host plasma membrane through which the mature IL-18 is secreted. The GSDMD pores eventually lead to the lysis or extrusion of infected cells, thereby eradicating infected cells from the intestinal epithelium. Previous studies established the role of *Shigella* T3SS effectors in the suppression of the activation of the CASP4 inflammasome. OspC3 directly inhibits CASP4 and IpaH9.8 targets guanylate-binding proteins (GBPs). GBPs encapsulate cytosolic *Shigella* to promote the activation of CASP4 inflammasome. To identify additional T3SS effectors that suppress epithelial cell death, I conducted an effector gain-of-function screen using a bottom-up platform (mT3Sf). From this, I found that cIpaH1, an E3-ubiquitin ligase, also inhibits mT3Sf-triggered host cell death. In follow-up studies, I found that in the context of *Shigella*, cIpaH1 cooperates with OspC3 and IpaH9.8 to suppress cell death. To identify host protein targets of cIpaH1, I developed ‘effector-BioID,’ a proximity labeling approach to identify protein targets of effectors delivered via the *Shigella* T3SS into infected cells. For cIpaH1, I identified zDHHC5, a palmitoyltransferase, as a target of cIpaH1. zDHHC5 was recently established to promote cell death via GSDMD palmitoylation, which is crucial for its membrane insertion and pore formation in macrophages. I have confirmed that cIpaH1 targets zDHHC5 for degradation and found that zDHHC5 is required for cIpaH1-mediated suppression of pyroptosis in the epithelial cells. Using an Acyl-PEG exchange assay to monitor the status of protein palmitoylation, I found that cIpaH1 inhibits GSDMD palmitoylation within *Shigella*-infected epithelial cells. My preliminary data also suggested that inflammasome activation promotes GSDMD palmitoylation in the *Shigella*-infected epithelial cells. This study not only identifies a novel mode of bacterial suppression of pyroptosis but also provides an example of how bacterial virulence factors cooperate to suppress host innate immune responses.

INDISPENSABLE COMPANIONS: MYCOVIRUSES AS ESSENTIAL COMPONENTS OF FUNGAL PATHOGENS

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Fungal pathogens pose a significant threat to global health. *Aspergillus fumigatus* accounts for approximately 65% of all invasive fungal infections in humans, with mortality rates from invasive aspergillosis reaching nearly 50%. Mycoviruses, viruses that infect fungi, can modulate fungal virulence in plant pathogenic fungi, leading to either hypovirulence or hypervirulence. However, their impact on fungal pathogenesis in mammals has remained largely unexplored. Here, utilizing an *A. fumigatus* strain naturally infected with Aspergillus fumigatus polymycovirus-1M (AfuPmV-1M), we found that the mycovirus confers a significant survival advantage to the fungus under conditions of oxidative stress, heat stress, and within the murine lung. Thus, AfuPmV-1M modulates fungal fitness, resulting in increased virulence and the progression of exacerbated fungal disease. Moreover, antiviral treatment reverses the virus-mediated increase in virulence, representing a promising "antipathogenicity" therapy against virus-bearing pathogenic fungi. Collectively, these findings reveal that mycoviruses act as pivotal 'backseat drivers' in human fungal diseases, underscoring significant clinical implications and offering promising avenues for novel therapeutic strategies.

THE ANTIFUNGAL PEPTIDE ENTV INHIBITS VIRULENCE BY REDUCING EXTRACELLULAR VESICLE RELEASE

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New antifungal agents with novel mechanisms of action are desperately needed. We have previously reported that a bacteriocin produced by *Enterococcus faecalis*, EntV, is a potent antivirulence agent that blocks adhesion and biofilm formation in *Candida albicans* in invertebrate and murine infection models. Structural studies and systematic peptide library screening have identified a 10 amino acid synthetic peptide, P4D, that is effective at reducing tissue fungal burden in oral and systemic models in mice, and prolongs survival after intravenous inoculation. P4D has activity against *C. auris* in mice, and against *Cryptococcus* and *Coccidioides* species in invertebrate models. Moreover, it is orally bioavailable in mice and is not toxic to mammalian cells. It is neither static nor cidal to Candida species, nor does it synergize with other stresses. Instead, it binds to the cell surface in dynamic punctae without specific stoichiometry. It colocalizes with dyes that preferentially stain extracellular vesicles (EVs), which are immunostimulatory and contain cargo important for extracellular matrix production. We screened a published panel of mutants that are known to reduce EV production in nematodes, mostly components of the ESCRT pathway, finding that these mutants generally were less sensitive to the peptide, but also attenuated for virulence. Direct treatment of *C. albicans* with EntV peptides reduces EV production ~8-fold, and the vesicles are more heterogeneous than those from untreated cells. Thus, the anti-virulence mechanism of this unusual peptide is associated with a reduction in EV release.

SYSTEMATIC DISCOVERY OF PIP-BINDING LEGIONELLA EFFECTORS REVEALS STRUCTURALLY CONSERVED MODULES IN BACTERIA

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Phosphoinositide (PIP) lipids regulate membrane identity and trafficking, making them key targets for intracellular pathogens. Identifying bacterial PIP-binding effectors has been challenging because they typically lack the canonical lipid-binding domains found in eukaryotes. Here, we use a multifaceted approach to systematically identify new PIP-binding effectors in *Legionella pneumophila*. We screened 241 His-tagged effectors using PIP-coated bead pulldowns and mass spectrometry, identifying 89 candidates. To refine this set, we generated mCherry-tagged constructs and assessed localization in HeLa cells co-expressing GFP-tagged PI(3)P or PI(4)P biosensors. Confocal imaging revealed enrichment at PIP-positive membranes, and biochemical assays confirmed direct binding for 18 effectors. Structural analysis revealed a shared compact alpha-helical fold, often within cryptic domains, which truncation assays showed to be both necessary and sufficient for PIP binding. Leveraging this structural signature, we identified and validated 12 additional effectors, bringing the total to 30 and more than doubling the known *Legionella* PIP-binding repertoire. Several had established roles in infection but were not previously linked to lipid binding, revealing an unrecognized facet of their function. Extending this structure-guided approach to *Coxiella* and *Burkholderia*, we identified virulence factors with conserved folds and confirmed their PIP-binding activity. Except for *Burkholderia* BopA, which bound PI(5)P, newly identified effectors from *Legionella* and *Coxiella* showed strong preference for PI(3)P, a lipid enriched on endosomal and autophagic membranes. This underscores PI(3)P targeting as a central strategy in their intracellular lifestyles. Our findings reveal a conserved helical PIP-binding module and highlight convergent evolution on phosphoinositide recognition as a widespread bacterial membrane-targeting mechanism.

COMBINING SINGLE-CELL RNA SEQUENCING AND LIVE-CELL IMAGING TO STUDY THE LIFE CYCLE OF INTRACELLULAR MICROSPORIDIAN PATHOGENS

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Microsporidia are an early-diverging group of fungal pathogens comprising over 1500 species that infect a wide variety of hosts ranging from insects to humans. As obligate, intracellular pathogens, microsporidia have evolved highly reduced genomes resulting in the loss of many regulatory and metabolic pathways, including the biosynthesis of amino acids, nucleotides, and lipids, driving these parasites to rely solely on their hosts for metabolites. Due to the lack of tools available for genetic manipulation in microsporidia, our understanding of how these parasites successfully replicate within host cells remains limited. Using single cell RNA sequencing (scRNA-seq) and live-cell imaging techniques, we are investigating the transcriptional and cellular dynamics of microsporidia infection. Our scRNAseq data explores how host cells respond to microsporidia infection, and also provides a blueprint for parasite development. The parasite transcriptome reveals a vast increase in the transcription of secreted proteins which may be important for spore wall formation, polar tube development, and exit from the host cell. On the host side, our results reveal that a small population of cells mount a response to microsporidia infection while most of the cells fail to detect invading parasites and are transcriptionally indistinguishable from uninfected cells. This suggests that microsporidia invades host cells without being detected allowing for successful parasite development. In parallel to our transcriptional studies, we are now using live-cell imaging to monitor parasite development on a cellular level. To overcome the lack of tools available for live-cell imaging in microsporidia, I have developed a technique termed "silhouette microscopy" which now allows us to investigate the replication dynamics of the microsporidian life cycle from entry into a host cell, all the way to exit from the host cell. I will discuss my work combining scRNAseq and live-cell imaging to study how microsporidia establish a replicative niche in host cells.

CRYPTOCOCCAL MORPHOGENESIS AND ANTIFUNGAL VACCINE DEVELOPMENT

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Cryptococcus neoformans is a ubiquitous free-living soil yeast and opportunistic pathogen that causes ~223,100 cases of cryptococcal meningitis per year, killing over 180,000 people. This fungus is responsible for 19% of deaths in AIDS patients and is fatal without treatment. Vaccination is one of the most effective public health measures for preventing and managing infectious diseases. However, developing effective vaccines against invasive fungal infections remains a scientific challenge and there is no clinically available vaccine against any invasive fungal disease. This is predominantly due to large antigenic repertoires, complicated life cycles, and the capacity of fungal pathogens to evade the host immune system. Additionally, antifungal vaccines often need to work for at-risk individuals who are immunodeficient. To develop vaccines against *Cryptococcus neoformans*, we took advantage of our previous observation that cryptococcal cells in the filamentous form elicit strong protective immune response. Here we identified antigens present in the filamentous form and explored their protective effect as vaccines either in the recombinant protein platform or the mRNA-lipid nanoparticle platform. Our recent results indicate strong protection of mRNA vaccines against cryptococcosis in mouse models. Our results reveal key scientific areas that need to be explored to actualize the development of effective antifungal mRNA vaccines.

ANTIBIOTICS ACCUMULATE TO THERAPEUTIC LEVELS IN *KLEBSIELLA PNEUMONIAE* LIVER ABSCESSES BUT FAIL TO ERADICATE ANTIBIOTIC TOLERANT POPULATIONS

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Liver abscesses caused by hypervirulent *Klebsiella pneumoniae* (hvKp) can lead to severe metastatic complications with up to 40% mortality. Even in the absence of antibiotic resistance, hvKp liver abscesses often respond poorly to treatment, sometimes requiring surgical resection. The reason for these poor outcomes remains unknown. Here, we established a novel hvKp wound model in outbred immunocompetent mice, which progresses to systemic infection and mature liver abscesses that were recalcitrant to frontline antibiotic therapy. Using a combination of quantitative and infrared matrix-assisted laser desorption electrospray ionization imaging mass spectrometry, we discovered that antibiotics fail to kill *K. pneumoniae* in liver abscesses, independently of resistance or spatial distribution of antibiotics, strongly implicating antibiotic tolerance in treatment failure. Notably, the inadequate antibiotic efficacy observed in our mouse studies mirrors clinical outcomes. These findings underscore the urgent need we need to determine the mechanism of antibiotic tolerance which may lead to novel therapeutic approaches.

MONITORING BACTERIAL HEME TRANSFER *IN VITRO*: A FLAsH-Y NEW APPROACH.

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Streptococcus pyogenes is an obligate human pathogen that poses a significant burden on human health. Heme serves as its primary iron source and is essential for infection and virulence. To acquire heme, *S. pyogenes* employs a series of surface hemoproteins that extract heme from the host hemoglobin (Shr), transport it across the bacterial wall (Shp), and import heme across the membrane (SiaA) for intracellular degradation. FlAsH is a fluorophore that specifically binds to a tetracysteine (TC) motif and exhibits fluorescent quenching in the presence of nearby heme. I**n this study, we leveraged the quenching ability of FlAsH to study heme transfer between streptococcal envelop proteins Shr, Shp-TC, and SiaA *in vitro.*** Under first order conditions, we demonstrated the rapid passage of heme from Shr to Shp via FlAsH quenching, and under the same conditions we showed heme passage from Shp to SiaA via the dequenching of FlAsH. For the first time, following quenching and dequenching of fluorescent signal, we were able to witness the sequential transfer of heme from Shr to Shp to SiaA in a single assay by mixing the three proteins together. We also used ELISA and identified that Shr and Shp form a stable complex. Further experiments are ongoing to determine whether SiaA participates in a potential ternary complex. In summary, this is the first demonstration of the use of FlAsH to monitor rapid, intermolecular heme transfer between streptococcal surface proteins. Collectively, our data support the proposed heme importation cascade (Shr → Shp → SiaA) in Streptococcus pyogenes and establish FlAsH as a high-resolution tool for studying heme trafficking in vitro.

ROLES OF THE MAP KINASE SAKA IN STRESS RESPONSES AND VIRULENCE OF *ASPERGILLUS FUMIGATUS*

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MAP kinase signaling pathways contribute to fungal pathogen survival when faced with host defenses. Fungal-leukocyte interactions are critical to the sterilizing immunity that clears inhaled *Aspergillus fumigatus* conidia in defense against invasive pulmonary aspergillosis; a life-threatening disease in individuals with impaired myeloid cell numbers or function. The fungal genome encodes two stress-activated Hog1/P38 MAPK orthologs, SakA and MpkC. Deletion of either or both can impact survival under osmotic or oxidative stress, and virulence in an immunosuppressed mouse model (Bruder Nascimento et al. 2016, Molec. Microbiol.). To test the function of SakA in immunocompetent mice and to follow its intracellular localization during stress responses, we deleted SakA and constructed a SakA:Gfp fusion in the CEA10 H2A:mRFP background. Deletion mutants (*sakA*) were impaired in survival following exposure to 5 mM H_2O_2, in growth on minimal medium amended with 1 M sorbitol, and produced fewer conidia than the parent wild type strain at 4 days after inoculation. Using a flow cytometry-based conidia cell death assay, we observed that histone H2A:mRFP fluorescence was lost faster in *sakA* than in the wild type during treatment with 10 mM H_2O_2, but paradoxically, labeling with the dead cell indicator Sytox Blue appeared more slowly. These *in vitro* phenotypes were all rescued in a mutant strain reconstituted by integration of a wild-type SakA copy. We are testing the function of SakA in immunocompetent mice challenged by intra-tracheal infection with conidia of wild type or the *sakA* deletion. SakA:Gfp is retained in the nucleus upon osmotic stress but sequestered in the cytoplasm by exposure to an antimicrobial plant phenolic, ferulic acid, as observed in a plant pathogen (Zuchman et al. 2025, submitted). Cytoplasmic sequestering during a host defense response could attenuate the pathogen's ability to survive stress. Alternatively, or in parallel, sequestering away from the nucleus could mitigate cell death of the pathogen resulting from sustained activation of the Hog1/P38 pathway. Our application of fungal genetics to the central roles of the SakA MAPK cascade in stress responses should contribute to understanding how leukocyte-generated stresses determine whether *Aspergillus fumigatus* can evade the innate immune response and cause invasive infections.

FRIENDS OR FRENEMIES? ENTEROHEMORRHAGIC E. COLI (EHEC) INTERACTIONS WITH THE GUT MICROBIOTA.

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The mammalian large intestine is inhibited by a dense and highly adapted microbiota. Enteric pathogens such as EHEC must monitor the chemical landscape of the gastrointestinal tract (GI) to find its colonization niche. A healthy microbiota is dominated by the Bacteroides Phyla, followed by Firmicutes and Proteobacteria (that can thrive in aerobic environments). EHEC senses many host and microbial-derived cues made available in the intestine by B. theta (fucose harvested from the mucus, succinate produced under gluconeogenesis, and degradation of pectin into galacturonate) to enhance its virulence. E. faecalis is a pathobiont that belongs to the firmicute Phyla and also promotes EHEC virulence both at the transcriptional and post-transcriptional levels. At the transcriptional level, the adenine secreted by E. faecalis is imported by EHEC and works to disrupt the Hha/H-NS repression of virulence genes. Specifically, it promotes expression of the locus of enterocyte effacement (LEE) pathogenicity island (PAI) that encodes a type three secretion system (T3SS) essential for EHEC's virulence. Post-transcriptional, E. faecalis expresses a protease (GelE) that processes the translocon of EHEC's T3SS to enhance effector secretion and formation of attaching and effacing lesions (the hallmark of EHEC's infection on enterocytes. Altogether, mechanistic studies on pathogen-microbiota interactions in the gut point towards complex interaction among microbiota membership and bacterial pathogenesis.

MECHANISMS OF VAGINAL COLONIZATION BY GROUP B STREPTOCOCCUS: IMPACT OF COMMENSAL FUNGI ON BACTERIAL VIRULENCE POTENTIAL

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The vagina hosts a rich microbiome that can include Group B Streptococcus (GBS), an opportunistic pathogen associated with adverse pregnancy outcomes and severe neonatal disease. Vaginal colonization is the critical precursor to these events, yet our understanding of how colonization is impacted by neighboring microbes in this environment is limited. Candida albicans (Ca) is co-isolated with GBS at rates of up to 55% and is an independent risk factor for GBS carriage. We have developed a murine model of Ca-GBS co-colonization and find that Ca promotes GBS persistence and survival in the vaginal lumen. To further our understanding, we performed transcriptomics analysis and identified key elements involved in interactions between GBS, Ca, and vaginal epithelial cells. For GBS, we observe differential regulation of distinct repertoires of virulence factors in each co-culture condition. Additionally, GBS induces Ca arginine biosynthesis, which is directly implicated in GBS virulence and persistence in vivo. Further, we show that direct binding between Ca and GBS promotes bacterial association to vaginal epithelia. Utilizing a panel of GBS vaginal isolates from pregnant individuals, we find that nearly all isolates exhibit high levels of co-aggregation with Ca. This suggests that the capacity to physically interact with Ca is highly conserved among clinically relevant lineages of GBS. Importantly, Ca alters the host antimicrobial response to GBS by downregulating epithelial-derived chemokines. Here, we show that transcriptional reprogramming and direct binding between Ca and GBS promote bacterial fitness in the host environment. Future work will involve investigating mechanisms of interaction and the host response during vaginal co-colonization *in vivo*.

LINEAGE TRACING AND THE QUANTITATIVE PROPERTIES OF BACTERIAL INFECTIONS

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Infections are the result of complex overlapping spatial and temporal processes of bacterial clearance, replication, and dissemination. Quantifying these processes is challenging with traditional measurements of infectious burden, typically the enumeration of colony-forming units, since such methods cannot monitor individual clones. This limitation can be overcome by the introduction of fitness-neutral genetic diversity through the genomic integration of short DNA barcodes. Through deep sequencing and quantification of the relative abundance of these barcodes, it becomes possible to map the trajectories of individual bacterial clones during infection, a process broadly known as lineage tracing. Experiments that leverage lineage tracing can define the underlying “quantitative properties” of infections. Such quantitative properties include identifying which clones give rise to the infectious population, defining where and when these clones disseminate, and measuring how these clones replicate or are cleared over time. Furthermore, performing lineage tracing in the contexts of host or bacterial perturbations can greatly enhance our understanding of how host and pathogen factors control infection. Here, we present a collaborative initiative towards defining generalizable and unique quantitative properties of bacterial infections. These findings synthesize observations from lineage tracing projects across several independent research laboratories, spanning experimental infection models with *Salmonella enterica*, *Pseudomonas aeruginosa*, *Yersinia pseudotuberculosis*, *Escherichia coli*, *Listeria monocytogenes*, and *Klebsiella pneumoniae*. We highlight two principles emerging from these studies: 1) Systemically circulating bacteria are consistently exchanged across specific compartments, yet in situ replication can arise when a specific niche can be occupied, such as within an abscess or in the gallbladder. 2) Innate immune responses reproducibly reduce the number of clones that initiate infection, and a direct consequence of this reduction is that a greater number of inoculated organisms are required to cause infection. We also describe a collaborative and experimental infrastructure aimed to increase accessibility of bacterial lineage tracing to the broader scientific community. Continued application of lineage tracing across diverse contexts has significant potential to define new quantitative properties of infectious diseases and deepen the mechanistic understanding of host and pathogen factors in infection.

CLOSTRIDIOIDES DIFFICILE PATHOGENESIS: FROM NURSERY TO NURSING HOME

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Early life is a critical developmental window during which a beneficial relationship between host and microbiota is established. Perturbations to this delicate ecosystem can disrupt host-microbiota symbiosis and immune homeostasis. The impact of pathogenic microbes on early life neonatal development and long-term health is still poorly defined. One of the most common pathogens in early life is *Clostridioides difficile*, which colonizes the majority of infants in the first two years of life. Despite being a major cause of severe gastrointestinal disease in adults, colonization with *C. difficile* is clinically asymptomatic in infants and thought to be largely benign. The impact of early life carriage of this toxin producing pathogen on neonatal health has not been explored. Here, we investigate the impact of early life *C. difficile* colonization and pathogenesis on neonatal development and long-term health.

PERSISTENT SALMONELLA INFECTIONS IN HUMANS ARE ASSOCIATED WITH CONVERGENT EVOLUTION IN THE BARA/SIRA REGULATORY PATHWAY

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Persistent bacterial infections are a significant public health concern, contributing to prolonged disease, treatment failure, and the emergence of antimicrobial resistance. While a number of clinically important bacterial pathogens, including *Salmonella enterica*, can cause persistent infections in humans, the underlying mechanisms are poorly understood. In this study, we analyzed 639 longitudinal *Salmonella* isolates from 256 patients with infections lasting 30 to 2,001 days. Whole-genome sequencing revealed widespread convergent evolution in global regulatory genes, most notably the BarA/SirA two-component system, across a wide diversity of non-typhoidal *Salmonella* serovars.

Mutations in *barA* and *sirA* accumulated during infection in nearly 10% of patients and were associated with consistent transcriptomic signatures: RNA-seq analysis showed reduced expression of virulence genes encoded on *Salmonella* Pathogenicity Islands (SPI) 1 and 4, which are required for epithelial invasion and enteritis. Using isogenic mutants in the *S.* Typhimurium SL1344 background, we confirmed that patient-derived *barA/sirA* mutations dampen virulence gene expression and reduce colonization in an acute mouse model of salmonellosis. Infected macrophages exposed to these mutants mounted weaker pro-inflammatory transcriptional responses, characterized by reduced cytokine and immune effector expression.

Conversely, in a murine model of persistent infection, *barA/sirA* mutants established long-term colonization and were shed at levels comparable to or exceeding wild-type strains at later stages of infection. These findings suggest that regulatory mutations reducing acute virulence enable immune evasion and persistence within the host. Importantly, nucleotide diversity analysis showed that *barA* and *sirA* are not mutational hotspots, supporting the idea that these changes are adaptively selected rather than stochastic. Together, our data highlight a novel strategy for bacterial persistence: downregulation of global virulence regulators facilitates chronic colonization through attenuation of immune-triggering pathways. This study not only illuminates a key mechanism underlying long-term *Salmonella* carriage in humans but also suggests broader relevance for other pathogens where regulatory rewiring supports persistent infection.

CANDIDA ALBICANS ENHANCES *STAPHYLOCOCCUS AUREUS* VIRULENCE WITH HOST SPECIES-SPECIFIC EFFECTS

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Colonization is a major risk factor for *Staphylococcus aureus* infection. Interactions with other microbes at colonization sites can regulate the transition of *S. aureus* from commensal to pathogen, which impacts disease onset and outcome. Fungi are frequently found interacting with bacteria at these sites, yet the ways that cross-kingdom interactions regulate bacterial virulence are incompletely characterized. Our overarching research goal is to define how the common co-colonizing fungus *Candida albicans* shapes the pathogenesis of invasive *S. aureus* infections. Most of our understanding of how *C. albicans* impacts *S. aureus* infection comes from studies with murine systems, yet several *S. aureus* toxins are highly selective for human cells. Therefore, we sought to delineate mechanisms by which *C. albicans*-*S. aureus* interactions regulate virulence by first testing cytotoxicity of co-culture supernatants towards human and murine cells. We determined that *C. albicans* enhances *S. aureus* cytotoxicity towards monocytes from both species via a mechanism requiring the Agr system, a major regulator of *S. aureus* virulence factors. Unexpectedly, we discovered that *C. albicans* induces significant cytotoxicity of a *S. aureus agr* mutant, but only towards human monocytes. This is surprising because *agr* mutants, which are commonly recovered from invasive and chronic human infections, are typically considered minimally toxic. We discovered that *C. albicans* activates *S. aureus* SaeRS, a two-component system that regulates many human-selective toxins, and that SaeRS is required for induced cytotoxicity of *S. aureus agr* mutants towards human cells. Using *S. aureus* SaeRS-regulated toxin mutants, we identified that cytotoxicity towards human monocytes requires the toxin Panton-Valentine Leukocidin (PVL), which selectively binds the human isoform of C5aR1. To determine how *C. albicans* activates SaeRS, we found that hyphae and candidalysin, which are two important *C. albicans* virulence factors, are not required for enhanced human cell death. However, *C. albicans* spent media is sufficient to induce *S. aureus* virulence, suggesting that a fungal secreted factor or media modification might activate SaeRS. Using *C. albicans* and non-*albicans* clinical isolates, we identified strains that activate Agr but fail to activate SaeRS-driven human cell cytotoxicity. This indicates that *Candida* activates *S. aureus* SaeRS and Agr by different mechanisms. Taken together, our data reveal that *C. albicans* enhances *S. aureus* virulence selectively towards human cells by activating two distinct virulence systems.

WIRED AND GUARDED: THE NEUROIMMUNE LANDSCAPE OF THE SKIN

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The interaction between the immune system and the somatosensory nervous system is essential for modulating itch sensation and maintaining survival. One hallmark of chronic itch is the hyperinnervation of the skin by sensory fibers, yet it is unclear what drives this aberrant nerve growth or how it contributes to disease progression. Here, we identify immunity to microbiota as a key trigger of sensory neuron plasticity and pruritus. In a murine model of psoriatic itch, we show that prior exposure to commensal *Staphylococcus aureus* followed by imiquimod treatment results in heightened skin inflammation, increased itch behavior, and marked hyperinnervation by CGRPα^+ sensory neurons. Accordingly, single-nuclei RNA sequencing of dorsal root ganglia reveals that microbiota-driven inflammation induces a regenerative transcriptional program in sensory neurons, including upregulation of axonal growth, injury response, and IL-17RA signaling pathways. Mechanistically, we demonstrate that IL-17A/IL-17RA signaling within TRPV1$^+$ sensory neurons, is both necessary and sufficient to drive hyperinnervation and pruritus. As such, targeted genetic deletion or antibody blockade of IL-17A/IL-17RA axis significantly reduces nerve fiber density, inflammation, and itch. These results establish a causal link between microbiota-induced immune responses and sensory circuit remodeling in the skin, highlighting sensory hyperinnervation as a pivotal contributor to chronic itch pathogenesis. Our findings provide a conceptual framework for targeting neuroimmune interactions, specifically sensory neuron hyperinnervation, as a therapeutic strategy for pruritic skin disorders, and suggest that sensory neurons are not just passive recipients of inflammation but active participants in disease amplification.

PHENOTYPIC LANDSCAPE OF A FUNGAL MENINGITIS PATHOGEN REVEALS ITS UNIQUE BIOLOGY

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Cryptococcus neoformans is the most common cause of fungal meningitis and the top-ranked W.H.O. priority fungal pathogen. Only distantly related to model fungi, *C. neoformans* is also a powerful experimental system for exploring conserved eukaryotic mechanisms lost from specialist model yeast lineages. To decipher its biology globally, we constructed 4328 gene deletions and measured-with exceptional precision--the fitness of each mutant under 141 diverse growth-limiting *in vitro* conditions and during murine infection. We defined functional modules by clustering genes based on their phenotypic signatures. In-depth studies leveraged these data in two ways. First, we defined and investigated new components of key signaling pathways, which revealed animal-like pathways/components not predicted from studies of model yeasts. Second, we identified environmental adaptation mechanisms repurposed to promote mammalian virulence by *C. neoformans*, which lacks a known animal reservoir. Our work provides an unprecedented resource for deciphering a deadly human pathogen.

ALLELIC VARIATIONS AND GENE CLUSTER MODULARITY ACT AS NON-LINEAR BOTTLENECKS FOR CHOLERA EMERGENCE

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The underlying factors that lead to specific strains within a species to emerge as human pathogens remain mostly enigmatic. The diarrheal disease cholera is caused by strains from a phylogenetically confined group within the *Vibrio cholerae* species, the pandemic cholera group (PCG), making it an ideal model system to tackle this puzzling phenomenon. Comprehensive analyses of over 1,840 *V. cholerae* genomes, including novel environmental isolates from this study, reveal that the species consists of eleven groups, with the PCG belonging to the largest and located within a lineage shared with environmental strains. This hierarchical classification provided us with a framework to unravel the eco-evolutionary dynamics of the genetic determinants associated with the emergence of toxigenic *V. cholerae*. Our analyses indicate that this phenomenon is largely dependent on the acquisition of unique modular gene clusters and allelic variations that confer a competitive advantage during intestinal colonization. We determined that certain PCG-associated alleles are essential for successful colonization whereas others provide a non-linear competitive advantage, acting as a critical bottleneck that clarifies the isolated emergence of PCG. For instance, toxigenic strains encoding non-PCG alleles of a) *tcpF* or b) a sextuple allelic exchange mutant for genes *tcpA*, *toxT*, *VC0176*, *VC1791*, *rfbT* and *ompU*, lose their ability to colonize the intestine. Interestingly, these alleles do not play a role in the colonization of newly stablished model environmental reservoirs. Our study uncovers the evolutionary roots of toxigenic *V. cholerae* offering a tractable approach for investigating the emergence of pathogenic clones within an environmental population.

POST-TRANSLATIONAL MODIFICATIONS REGULATE *STAPHYLOCOCCUS AUREUS* RESISTANCE TO HOST OXIDATIVE STRESS

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The innate immune system controls bacterial infection through the coordinated actions of phagocytic leukocytes. These cells generate large amounts of antimicrobial free radicals, including reactive oxygen species (ROS), via the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in a process known as respiratory burst. NADPH oxidase dysfunction renders individuals highly susceptible to infection with bacteria and fungi, underscoring the relevance of respiratory burst to infection and its role as a potent antimicrobial. Bacterial pathogens have adapted in several ways to evade the detrimental e"ects of respiratory burst. For example, *Staphylococcus aureus* activates cellular repair pathways and synthesizes enzymes and small molecules that neutralize ROS. In addition to neutralizing ROS, other small molecules play crucial roles in regulating intracellular redox homeostasis. Low-molecular-weight thiols like coenzyme A and bacillithiol protect sulfur-containing proteins by interacting with their thiol groups, mitigating damage and facilitating the recovery of enzymatic function after stress. Thus, the bacterial response to oxidative stress entails both neutralizing ROS and preventing oxidation of preexisting cellular components. Here, we found that the lipoic acid carrier protein, GcvH-L, is required for *S. aureus* to resist oxidative stress. GcvH-L is encoded within an operon that is conserved in several pathogenic microorganisms. The operon also encodes LplA2, a redox-responsive lipoic acid ligase, and SirTM, an ADPribosyltransferase. We demonstrate that ADP-ribosylation of lipoyl-GcvH-L protects the sulfhydryls of lipoic acid from oxidation and regulates its transfer from GcvH-L to enzyme complexes that are required for central metabolism. A Δ*gcvH-L* mutant is attenuated during infection and is more sensitive to phagocyte respiratory burst, phenotypes that are abrogated with NADPH oxidase deficient mice. Thus, ADP-ribosylation and lipoylation converge on GcvH-L to promote *S. aureus* resistance to oxidative stress. Altogether, we propose that the components of the lplA2 operon constitute a redox-sensitive molecular switch that responds to the oxidative state of the cell to protect lipoic acid from oxidative damage and promote rapid cellular recovery by regulating the delivery of reduced lipoic acid to metabolic enzymes.

EPIMUTATIONS EVOKE TRANSIENT ANTIMICROBIAL DRUG RESISTANCE

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Microorganisms evolve via sexual/parasexual reproduction, mutators, aneuploidy, Hsp90, or prions. Mechanisms that are detrimental can be repurposed to generate diversity. Microbes are known to evolve resistance to antimicrobial agents (AMR) via pathways involving both stable and unstable genetic mechanisms, such as aneuploidy underlying azole resistance in *Candida* and *Cryptococcus*. We discovered a new mechanism conferring antifungal drug resistance in the human fungal pathogen *Mucor*. Spontaneous resistance to the antifungal drug FK506 was found to evolve via two distinct mechanisms. One involves Mendelian mutations in the known drug targets (FKBP12, calcineurin A or B) conferring stable drug resistance. The second occurs via epigenetic processes involving RNAi or ectopic heterochromatin (with or without RNAi) resulting in unstable, transient drug resistance. In murine infection models both RNAi-dependent and heterochromatin epimutants were found to be largely stable throughout the course of infection. Recent studies reveal RNAi epimutations are inheritable following sexual reproduction and meiosis. These studies uncover a novel, reversible, transient epigenetic epimutation mechanism controlling phenotypic plasticity, with implications for antimicrobial drug resistance, in vivo mechanisms of pathogenesis, and RNAi-regulatory mechanisms in fungi and other eukaryotes. These studies reveal inheritable genetic information transmitted by RNA, which may reflect facets of an RNA world hypothesized to have existed during the origins of life, predating the evolution of DNA as the central conduit of inherited genetic information. The full impact of epimutations in this and other genetic systems and species may have eluded discovery previously given their inherently unstable nature.

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EVOLVED ADAPTATIONS TO TOLERATE ANTIBIOTICS BY ALTERING THE RNA DEGRADASOME

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To understand how antibiotic- and immune state-driven selective pressures lead to antibiotic treatment failure and the emergence of tolerance and antibiotic resistance, we evolved *Streptococcus pneumoniae* in mice of differing immune states that were continuously exposed to increasing amounts of various antibiotics. Genetic and phenotypic characterization of 90 lineages evolved through over 15 repeated infections revealed the dynamics of adaptive evolution as functions of antibiotic pressure and host immunity. Surprisingly, true resistance was rare owing to fitness costs of these mutations, but most evolved populations from all immune states developed antibiotic tolerance. One frequent and independently mutated gene is *rny*, encoding RNAse Y that is a major element of the RNA degradasome. These mutations were exclusively single nonsynonymous mutations that rose to high frequency, indicative of strong selection for subtle changes in this highly conserved gene. We reconstructed these SNPs in the ancestral strain and found they phenocopied the evolved populations with both tolerance to several antibiotics and a decreased refractory period following antibiotic exposure, enabling shorter lag phase and faster population recovery. Comparison and integration of both single cell RNA-Seq and global RNA-degradation analyses revealed that antibiotic exposure causes accelerated genome-wide transcription that is followed by a population crash, rapid uncontrolled RNA degradation, and death. In contrast, *rny* mutants confer antibiotic tolerance, cause instantaneous and selective degradation of transcripts upon antibiotic exposure with those encoding for essential cellular processes being prioritized. Importantly, while RNA content is reduced ~70-fold rapidly following antibiotic exposure in these mutants, the RNA that remains is of high quality, which promotes bacterial survival and more rapid recovery from lag phase following antibiotic removal. Using experimental evolution as a powerful genome-wide forward genetic screen, we discovered that pneumococcal cell death from antibiotics is mediated by failures in the RNA degradasome that can be remedied by single mutations. In the wild-type strain antibiotics cause uncontrolled transcription followed by massive global transcript degradation that is reminiscent of eukaryotic programmed cell death pathways, but mutants that modulate this pathway can promote antibiotic treatment failure.

TINKERING WITH TIME: IN-HOST EVOLUTION OF XDR MYCOBACTERIUM AVIUM REVEALS HYPERMUTABILITY AS AN ADAPTIVE MECHANISM DURING CHRONIC LUNG INFECTION

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Mycobacterium avium (MAC) is an opportunistic pathogen that can cause severe pulmonary disease in patients with cystic fibrosis (PwCF). During infection, MAC is exposed to a variety of environmental stressors that shape its evolutionary trajectory. While PwCF can be chronically infected with MAC for years, how these bacteria adapt and persist during infection have yet to be explored. Here, we conducted a retrospective, longitudinal whole genome sequencing (WGS) study on a single PwCF with chronic MAC infection for over 5 years to identify important genotypic and phenotypic changes required for adaptation. Phylogenetic analysis revealed the emergence of two clonal populations, with the later-emerged lineage being characterized by higher rates of single nucleotide polymorphisms (SNPs) and non-synonymous mutations found in genes associated with cell wall modification, antibiotic resistance, oxidative stress survival, and DNA damage repair. Mutations in the DNA repair genes, uvrA and recR, were associated with multi-drug resistance and elevated mutation rates when we tested the clinical MAC isolates. These findings suggest that these DNA repair mutations are potentially implicated in a mutator phenotype, where loss of DNA repair results in an increased mutation rate to produce more genetic variants better adapted to a stressful environment. To test this hypothesis, we generated isogenic genetic mutants of M. smegmatis and examined their ability to repair DNA damage and increase mutagenesis. RecR G4A was associated with increased susceptibility to DNA damage, while UvrA T45I was associated with elevated mutation rates. These differences in phenotypic traits suggest a potential dual gene mutator with interplay between nucleotide excision repair and recombination repair. Overall, these findings not only highlight the importance of hypermutability in the evolution of CF pathogens but provides novel targets for diagnostics and therapeutic intervention of MAC lung disease.

ANALYSIS OF ADHESION REGULATION IN CANDIDA AURIS

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Microbial communities must be able to respond to dynamic changes in their environment. One strategy is to be able to rapidly switch phenotypes, using a strategy called Bet Hedging, where an isogenic strain stochastically expresses a specific phenotype leading to population heterogeneity. This results in some maladapted individuals, but these fitness tradeoffs are compensated by the selective advantage of other individuals during rapid environmental shifts. This phenomenon has been well studied in bacterial populations, especially in the context of persister cells during antibiotic treatment or the development of spores. However, this has not been well examined in mechanistic detail in fungal pathogens.

For Candida auris, an emerging fungal pathogen, a critical and virulence-associated phenotype is the ability to adhere and form a biofilm. However, the ability to strongly bind to a surface comes at the cost of being able to release, suggesting potential fitness tradeoffs between adhesion and transmission. Our previous work identified that the Scf1 adhesin is required for attachment to surfaces. We also observe that the expression of Scf1 in an isogenic population is heterogenous, although the ratio of high and low expressing cells appears to be heritable. Moreover, only the high Scf1-expressing cells in a population can adhere.

Here, we demonstrate that C. auris uses a combination of chromatin remodelers and the Efg1 transcription factor to control the proportion of cells that adhere to an abiotic surface. This leads us to propose a model in which there is epigenetic tuning of bet hedging, resulting in alterations in the proportion of cells that are adherent.

MYCOBACTERIUM TUBERCULOSIS PHOP IS REQUIRED FOR ALDEHYDE RESISTANCE IN MICE

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The human pathogen *Mycobacterium tuberculosis* primarily infects macrophages. Upon activation by IFNγ, infected macrophages increase production of the antimicrobial effector methylglyoxal, an aldehyde byproduct of aerobic glycolysis. To test if resistance to this aldehyde is important during infections, we performed a transposon mutagenesis screen for methylglyoxal sensitive mutants. The most sensitive mutants we identified had disruptions in *phoP*, an established transcriptional regulator of virulence. We found the *phoP* mutant was more susceptible to methylglyoxal because of its loss of robust membrane impermeability. While it is established that *phoP* strains are highly attenuated, we nonetheless found a *phoP* mutant was even more attenuated in mice that accumulate methylglyoxal. Collectively, our data show that a major function of *M. tuberculosis* PhoP is to provide resistance to methylglyoxal toxicity during infections.

VISUALIZING INTESTINAL COLONIZATION BY *VIBRIO CHOLERAE* USING MIPACT-HCR

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Acute intestinal infections like those caused by *Vibrio cholerae* typically alter the gut environment in drastic ways, displacing the resident microbiome. However, recent work has identified a commensal bacterial species called *Paracoccus aminovorans* which was found to be more abundant in people with active *V. cholerae* infections than in those without. In in vitro experiments, *P. aminovorans* was able to induce biofilm formation by *V. cholerae*, suggesting a mutually beneficial relationship during infection. Furthermore, mice co-infected with both *P. aminovorans* and *V. cholerae* have significantly higher burdens of *V. cholerae* than when treated with *V. cholerae* alone, suggesting a potential role for biofilms during infection. The enhanced colonization by *V. cholerae* requires biofilm matrix production, but, surprisingly, does not require the production of surface adhesins. We hypothesize that, while adhesive mutants may not show a colonization defect, they might have altered spatial localization within the mouse intestine.

To test this hypothesis, we have adapted a tissue-clearing protocol called Microbial Identification After Passive Clarity Technique (MiPACT). Unlike traditional histological techniques, MiPACT produces optically transparent tissues which can be imaged in three dimensions. By combining MiPACT with hybridization chain reaction-fluorescence in situ hybridization (HCR-FISH), we can visualize the native location of colonizing bacteria across the length of the infected mouse intestine. We also included the use of fluorescently-labelled wheat-germ agglutinin (WGA), which binds N-acetylglucosamine (GlcNAc) and sialic acid residues present in intestinal mucus, facilitating its visualization. Using these methods, we have recapitulated in vitro data in our dual *P. aminovorans/V. cholerae* infection model. Animals colonized with *V. cholerae* alone show low-level basal colonization, while dual-infected animals show a drastic increase of *V. cholerae* cells in both the intestinal crypts as well as the tips of the villi. However, dual-colonized animals that receive a biofilm matrix mutant *V. cholerae* strain do not show the same colonization enhancement, indicating that the in vitro relationship between P. aminivorans and *V. cholerae* is maintained in vivo. Ongoing work includes efforts to develop HCR-FISH probes for the detection of biofilm genes. This will allow us to determine whether colonizing *V. cholerae* cells are actively producing biofilms.

ADAPTING A QUANTITATIVE APPROACH TO ASSESSING AND MIMICKING MICROBIAL PHYSIOLOGY IN HUMAN CHRONIC WOUND INFECTION MODEL

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Laboratory models provide tractable, reproducible systems that have long served as foundational tools in microbiology. However, the extent to which these models accurately mimic the biological environments they represent remains poorly understood. In 2020, Cornforth et al. introduced a quantitative framework leveraging RNA sequencing to assess how well laboratory models capture microbial physiology in situ. Despite the significance of this framework, its application has been limited to characterizing the physiology of a single species in human infections, leaving a gap in our understanding of microbial physiology and overall community dynamics in polymicrobial contexts. Here, we adapted this quantitative framework to evaluate the accuracy of laboratory model systems in capturing the collective microbial community functions in polymicrobial infections. As a proof of concept, we apply this adapted framework to a polymicrobial model of chronic wound (CW) infection. Chronic wounds are wounds that fail to heal over a prolonged period and are often colonized by diverse bacterial species with varied metabolic capabilities and support the establishment of a range of microbe-microbe interactions that impact bacterial survival and disease progression. Despite this complexity, existing research has predominantly relied on single-species or pairwise models, which do not adequately represent the microbial diversity and functional dynamics observed in clinical CW infections. This limitation hinders the translation of laboratory findings to clinical relevance. To address this gap, we applied the quantitative framework to the four-member CW infection model developed by Sun et al as a test case. Our analysis demonstrates that the framework can be adapted to polymicrobial systems. Building on our prior work in large-scale metagenomic and metatranscriptomic analysis, we are now evaluating modifications to the host environment and community composition of the model to better capture the taxonomic and functional complexity of human CW infections. This approach will support the development of ecologically relevant CW infection models and the development of better treatment strategies.

GENOMIC ANALYSIS OF COMMUNITY-ASSOCIATED QUINOLONE-RESISTANT AND ESBL-PRODUCING *E. COLI*

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Background: Antimicrobial resistance of *Escherichia coli* to quinolones and extended spectrum beta-lactams (ESBLs) each pose a significant threat for development of gastrointestinal complications and extraintestinal infections. This resistance has contributed to the persistence of hyper-virulent *E. coli* sub-lineages H30R and H30Rx, which both belong to sequence type (ST) 131. ST131, is a common colonizer of the enteric gut has been extensively studied in healthcare settings, however its pervasiveness in a community setting is less understood.
Methods: This work includes a novel sample of 75 community-associated (CA) *E. coli* collected from 64 patients who experienced minimal healthcare exposures over the 12 weeks prior to receiving care at Barnes Jewish Healthcare (BJH) in St. Louis, Missouri. Accordingly, *E. coli* was cultured from stool samples selected for ciprofloxacin resistance and ESBL production, then whole genome sequenced. Core-genome SNP analysis identified multiple patients who carried multiple strains of drug-resistant *E. coli*.
Results: Genomic analysis identified 36/75 isolates belonging to ST131. Here, we observed a significant difference in the reported resistance genes among ST131 isolates compared to non-ST131 strains. Notably, all 36 ST131 isolates carried mutations in quinolone resistance determining regions, majority of which belong to the H30R sub-lineage. To evaluate how these isolates persisted over time, we utilized a published sample set of 88 ESBL-producing clinical isolates recovered from bloodstream and urinary tract infections recovered at BJH. When compared to CA *E. coli* cultivated on ESBL CHROMagar we identified 11 unique strain networks that persisted across varying body sites throughout the four-year study window.
Conclusion(s): Our findings support ST131 being a pervasive lineage whose global predominance poses a threat in both the community and clinical settings. Isolates derived from these two settings within the same timeframe revealed minimal differences in total ARG carriage and the reported ESBL genotypes, further highlighting the growing concern that drug-resistant *E. coli* is well established within the community and is not confined to the hospital setting.

EXPLORATION OF THE MYCOBACTERIAL PUTATIVE VIRULENCE FACTOR MPT70

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Mycobacterium tuberculosis (*M. tb*), the main causative agent of tuberculosis (TB), belongs to the *Mycobacterium tuberculosis* complex (MTBC), a group of closely related mycobacteria that cause TB in mammals. Other members include the animal-adapted species *Mycobacterium bovis* and *Mycobacterium orygis*. Despite sharing >99% identity, MTBCs differ in host specificity and virulence, in part due to mutations that alter protein expression.
This is the case of MPT70, a non-canonical virulence factor. *M tb*. expresses MPT70 at low levels *in vitro*, with inducible expression during macrophage infection. The host cue for this induction is unknown but is mediated by a positive regulator, sigma factor K (SigK) and a negative regulator, the regulator of sigma K (RskA). In *M. bovis*, two missense mutations in *rskA* result in constitutive MPT70 production. *M. orygis* also expresses MPT70 constitutively due to a read-through mutation (X233S) in *rskA*.
Murine infection studies in our lab have shown that *M. bovis* and *M. orygis* are significantly more virulent than *M. tb.*, with median survival times of 24-31 days compared to over 30 weeks for *M. tb*. To test whether this virulence phenotype is linked to MPT70 levels, *mpt70* was disrupted in both organisms. Disruption attenuated virulence in *M. bovis* but not in *M. orygis*, suggesting that the role of MPT70 may vary between species.
The role of MPT70 in virulence remains unknown. Based on the demonstrated role of MPT70 on *M. bovis* virulence and MPT70 upregulation in the virulent strain *M. orygis*, the lab has generated mycobacterial strains to better understand its function across species. The ORBIT (oligo-mediated recombineering followed by Bxb-1 integrase targeting) system has been employed to generate *mpt70* deletion strains in *M. tb*, *M. bovis*, *M. orygis*, and BCG Russia. Alternatively, the related organism *Mycobacterium marinum* can be employed in zebrafish (*Danio rerio*) infection models. *M. marinum* has a conserved SigK regulon and an *mpt70* homolog. Isogenic *M. marinum* strains with no and high *mpt70* expression have been generated and validated using ORBIT. The recombineering system pNIT:ET will be used to insert the X233S mutation in *M. marinum* for *mpt70* constitutive expression. These strains will be coupled with fluorescent techniques to explore the role of MPT70 in virulence using transparent zebrafish larvae.

THE ROLE OF AN ANNOTATED TRANSCRIPTION FACTOR AND A TRIPARTITE EFFLUX PUMP IN ANTIMICROBIAL RESISTANCE IN *BURKHOLDERIA THAILANDENSIS*

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The genus *Burkholderia* contains more than 100 species, including opportunistic human pathogens, facultative intracellular pathogens, and phytopathogens. *Burkholderia thailandensis* (BTH) is a surrogate for studying the host-pathogen interactions, virulence mechanisms and antimicrobial resistance within the *Burkholderia* genus. *Burkholderia* species are highly resistant to a plethora of antimicrobial agents. In the Gram-negative *Burkholderia* spp., efflux pumps play a central role in resistance mechanisms by exporting antimicrobial substances. Previously our lab has reported that members of a family of transcription factors known as Multiple Antibiotic Resistance Regulator (MarR) transcriptionally regulates both efflux pump genes and virulence-associated genes in BTH. There are 12 annotated MarR in BTH, but only few of them are characterized.

An annotated operon comprising *BTH_I2558-I2561* genes was characterized. *BTH_I2558* encodes a MarR transcription factor, which we named CwiR based on inferred roles in maintenance of cell wall integrity, and the other three genes (*BTH_I2559-61*) encode a tripartite efflux pump including an outer membrane protein, a periplasmic protein, and an inner membrane transporter protein. *BTH_I2558-61* was confirmed to be an operon using cDNA synthesis and polymerase chain reaction (PCR) techniques. Real-time quantitative PCR (RT-qPCR) techniques and mRNA sequencing were carried out for gene expression analysis at the transcriptional level. Proteomic analysis was conducted using quantitative mass spectrometry for analysis at the protein level.

Using a Δ*cwiR* strain in which *BTH_I2558* was disrupted, we found that CwiR suppresses the expression of the three adjacent efflux pump genes. Upregulation of these genes would be predicted to correlate with more antibiotics or other toxic compounds being pumped out of the bacterial cell. Unexpectedly, the Δ*cwiR* strain exhibited increased sensitivity to all tested antibiotics, suggesting that CwiR not only regulates the adjacent efflux pump genes but also influences additional genes. RNAseq analysis of the Δ*cwiR* strain revealed that CwiR serves as a master regulator that regulates 273 genes, including several involved in cell wall integrity. Elevated levels of proteins associated with structural maintenance of the cell wall were detected in the Δ*cwiR* proteome. Therefore, our data suggest that CwiR regulates antibiotic sensitivity by modifying cell wall integrity in BTH. This knowledge will aid in the development of new therapeutic interventions for multidrug resistant pathogenic Burkholderia species.

EPITHELIAL YAP SIGNALING CONTROLS EPITHELIAL–IMMUNE CROSSTALK IN INTESTINAL IMMUNITY

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Immune surveillance in the intestinal epithelium is tightly regulated through intricate interactions among epithelial cells, immune cells, and the gut microbiome. This dynamic environment is vital not only for sustaining tolerance to dietary antigens and commensal microbes but also for defending against pathogens, preventing oncogenic transformation, and limiting tissue damage. Although the transcriptional coactivator YAP has been recognized as a key regulator of intestinal regeneration, its role in coordinating immune-epithelial interactions and immune defense against intestinal pathogens has remained largely unexplored. In this study, we demonstrate that epithelial YAP is essential for the recruitment and maintenance of intraepithelial $CD4^+$ and $CD8^+$ T cells under homeostatic conditions. Mechanistically, we identified epigenetic pathways regulated by epithelial YAP signaling that drive immune-related transcriptional networks essential for maintaining immune homeostasis in the intestine. Loss of epithelial YAP impairs immune responses to Salmonella enterica and Listeria monocytogenes, resulting in heightened neutrophilia and reduced antigen-specific T cell responses, respectively. Together, our findings reveal a previously unrecognized role for YAP in orchestrating immune surveillance within the intestinal barrier. These findings underscore epithelial-intrinsic mechanisms as key regulators of host immune responses in barrier tissues across both health and disease states.

SEX INFLUENCES PROPOFOL IMMUNOSUPPRESSION DURING *KLEBSIELLA PNEUMONIAE* LUNG INFECTION

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Klebsiella pneumoniae (*K. pneumo*), a gram-negative bacterium and common gut colonizer, is a leading cause of hospital-acquired pneumonia with high rates of antibiotic resistance and mortality. *K. pneumo* can be acquired via invasive hospital procedures or during mechanical ventilation, and the use of sedation in these circumstances is associated with a higher risk of infection. Our lab has shown that propofol sedation, the most common drug for anesthetic induction, increases susceptibility of female mice to *K. pneumo* lung infection in comparison to ketamine, another intravenous sedative. Although propofol is rapidly cleared from the bloodstream, propofol urine metabolites are recovered several days after infusion and our experimental evidence suggests that a propofol-derived metabolite may mediate propofol-triggered immunosuppression. Based on recognized variations in male and female propofol metabolism, with female mice expressing increased levels of enzymes that metabolize propofol, we hypothesized a sex-linked difference in infection outcome after propofol sedation such that females would show greater susceptibility to propofol metabolite-mediated immune suppression. Differences in host immune response to infection dependent on sedative choice were compared by enumerating bacterial burdens in infected organs and quantifying cytokine differences in the lungs of animals sedated with either ketamine or propofol. Interestingly, under propofol but not ketamine sedation, mice exhibited a sex-dependent increase in the disseminated spread of *K. pneumo* to livers and spleens with female mice exhibiting significantly increased levels of bacterial dissemination. Our results are consistent with the hypothesis that a propofol metabolite leads to the suppression of innate immune responses that help to resolve microbial infection, and that the more rapid metabolism of propofol by females results in a worse outcome for infection. As anesthesia is a risk factor for nosocomial infections, these results indicate that sex-dependent changes underlying increased susceptibility to *K. pneumo* and other nosocomial pathogens following propofol exposure may warrant consideration of alternative sedation methods in health care settings.

DISSEMINATION OF *SERRATIA MARCESCENS* FROM THE LUNG VIA THE LYMPH NODE DURING BACTEREMIC PNEUMONIA.

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Pneumonia is a serious health concern that also provides a gateway for the systemic dissemination of bacterial pathogens. Accordingly, lower respiratory infections are the most frequent cause of sepsis-related deaths. *Serratia marcescens* commonly causes both pneumonia and bacteremia and is responsible for more than 100,000 global deaths each year. Our work has characterized several critical factors that enable *S. marcescens* survival and pathogenesis during bacteremia and colonization of peripheral organs. However, the processes by which *S. marcescens* escapes the lung and gains initial access to the bloodstream are unknown. *S. marcescens* instilled into the lungs of healthy mice enter circulation and are observed at high abundance in the spleen, liver, and kidneys less than 24 hours after inoculation, suggesting a rapid and efficient dissemination mechanism. Invasion of the endothelium and lymphatic transit are two potential routes that could facilitate this dissemination. To initially evaluate each, bacteria were quantified from blood and the lung-draining posterior mediastinal lymph node of infected animals. As early as four hours post-inoculation, bacteria (10^1-10^3 CFU) were routinely recovered from the lymph node (n = 8/10) while fewer animals (n = 4/10) had CFU above the limit of detection in the blood. The spleen, kidney, and liver were also colonized at this time, indicating that systemic dissemination had occurred. Notably, significant bacterial proliferation in the lung was not observed by four hours. Lymphatic bacteria were also quantitated from a non-draining site as a control, with no bacteria (detection limit = 1 CFU) observed in inguinal lymph nodes (n = 0/5). The ShlA hemolysin and capsule polysaccharide are both potent *S. marcescens* virulence factors that contribute to pathogenesis. Mice infected with an Δ*shlA* null mutant had significantly fewer bacteria recovered from the lung-draining lymph node compared to wild-type infected mice, indicating that lymph node localization is a bacteria-mediated process and that ShlA activity contributes. However, a capsule mutant was recovered from the lymph node at similar levels to wild-type bacteria, despite a dramatic fitness defect of acapsular bacteria in the lung and peripheral organs at later time points. Together, these results demonstrate that *S. marcescens* can disseminate from the lungs via the lymphatic system and ongoing work aims to further characterize this route at the levels of lung escape and lymphatic survival.

HETEROGENEITY IN VIRULENCE FACTOR EXPRESSION DURING *STAPHYLOCOCCUS AUREUS*-NEUTROPHIL INTERACTION

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Staphylococcus aureus is the leading cause of bacteremia worldwide and is associated with significant mortality due to increasing levels of antibiotic resistance and the lack of an effective vaccine. Systemic infection by *S. aureus* also results in the formation of kidney abscesses, which are difficult to eliminate with antibiotics even when strains are susceptible. Additional therapeutics are urgently needed, and *S. aureus* virulence factors represent attractive targets. However, a previous study in our lab demonstrated that activity of a master regulator of virulence factor expression, the *S. aureus* exoprotein expression (Sae) system, is heterogeneous over the course of kidney abscess development and among individual bacterial cells. Specifically, using a fluorescent *sae* reporter in *S. aureus* USA300 strain LAC, we showed that neutrophil-associated *S. aureus* exhibits a heterogeneous ON/OFF phenotype. Here, we further investigate this phenotype using human neutrophil models: HL-60 cell lines and primary neutrophils, both of which harbor intracellular *S. aureus* displaying heterogeneous Sae activity. We hypothesize that these phenotypes reflect distinct intracellular localizations of *S. aureus* within neutrophils (vacuolar versus cytoplasmic) which differ in pH, reactive oxygen species (ROS), and antimicrobial peptide exposure, influencing Sae activity. Individual bacteria may face distinct fates of survival, replication, bacterial killing, or even killing of the neutrophil, since leukocidin expression is controlled by Sae. To test this, we will determine colocalization with vacuolar markers and assess bacterial outcome. We will also determine whether Sae-ON bacteria promote neutrophil lysis via increased expression of Sae-regulated leukocidins using *hlgAB* and *hlgCB* leukotoxin reporters. Finally, we will compare isogenic LAC strains harboring the wild type $saeS^{L}$ allele, a constitutively active $saeS^{P}$ allele (from strain Newman), and a Δ*sae* deletion mutant to assess how Sae heterogeneity affects infection outcomes. Taken together, this study aims to uncover drivers and outcomes of differential virulence gene expression in *S. aureus*, providing insights for improved therapeutic strategies.

UNDERSTANDING REGULATION OF AGGR VIRULENCE BY FATTY ACIDS: A QUEST FOR ANTIVIRULENCE MOLECULES

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About 1.7 million deaths from diarrheagenic diseases occur annually worldwide, with roughly 525,000 occurring in children under five. Diarrhea typically results from infections in the intestinal tract caused by various pathogens, including strains of Escherichia coli (E. coli). Our focus is on enteroaggregative E. coli (EAEC), which carries a plasmid encoding various virulence factors, including aggR. AggR is a transcriptional regulator of virulence genes, including the attachment adherence fimbriae (AAF) essential for binding to human intestinal cells.

Fatty acids inhibit AraC/XylS family members from various pathogens, including Rns. Given that Rns and AggR are about 67% homologous, it was thought that AggR would be inhibited by fatty acids in a similar manner as Rns. Surprisingly, AggR shows different fatty acid sensitivity than Rns in reporter assays. Given the shared conserved residues with Rns, we hypothesize that the intrinsic properties of AggR, outside of the binding pocket, affect ligand binding specificity and influence the effectiveness of fatty acid inhibitors.

To test our hypothesis, we need to develop a high-throughput assay to be able to measure and quantitate AggR DNA binding. We have developed a fluorescence anisotropy assay and show that purified AggR specifically binds to a labeled CS3p probe and that binding can be outcompeted by medium- and long-chain fatty acids.

IDENTIFYING CONSERVED AND STRAIN-SPECIFIC FITNESS DETERMINANTS OF ETEC WITHIN THE GUT

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Enterotoxigenic Escherichia coli (ETEC) strains cause an estimated 220 million cases of diarrhea annually, resulting in the deaths of about 44,400 infants and young children. ETEC infections typically occur by ingesting contaminated water or food, with higher incidences in resource-limited countries. A significant obstacle in developing therapeutics against ETEC is its genetic heterogeneity. ETEC strains can vary significantly in their genomes, including their repertoire of plasmid-borne virulence factors that promote host colonization and diarrhea. These virulence factors include the secreted enterotoxins, heat-labile (LT) and heat-stable (ST) toxins, as well as over 27 outer membrane adhesive fimbrial or afimbrial colonization factors (CFs). Individual ETEC strains can express varying combinations of the LT and ST toxins and typically encode 1 to 3 different CFs. Much of our current understanding of ETEC pathogenesis is based on two reference strains, H10407 and E24377A, which do not capture the full diversity of this pathovar. To gain a more complete understanding of the phenotypic diversity of ETEC, we used a panel of in vitro assays and a high-throughput larval zebrafish gut colonization model to compare a cohort of nine fully sequenced ETEC strains representing the seven modern lineages of ETEC that are currently in global circulation. We found that the ETEC strains displayed distinct biofilm and swim motility phenotypes and also varied markedly in their resistance to reactive oxygen and nitrogen species, which are often encountered within host environments. All of the tested ETEC strains stably colonize and replicate within the zebrafish gut and effectively outcompete a non-pathogenic commensal E. coli strain. Deletion of key prototypical CFs, hypothesized to promote ETEC adherence to mammalian host cells, significantly impaired ETEC colonization within the zebrafish gut, validating the utility of the zebrafish host as a tool for assessing important ETEC fitness determinants. Following up on these observations, we are utilizing the zebrafish infection model with the cohort of ETEC isolates and Random Barcoded Transposon Sequencing (RB-TnSeq) to gain a more comprehensive understanding of both conserved and unique bacterial fitness determinants that are critical for ETEC colonization and survival within the gut. This approach allows us to overcome bottleneck issues often associated with TnSeq screens and, by revealing common mechanisms used by diverse ETEC strains to colonize the host, will inform the development of improved and more broadly effective therapeutics.

IN VIVO CHARACTERIZATION OF MICROBE HOST INTERACTION BETWEEN *STAPHYLOCOCCUS AUREUS* AND THE MAMMALIAN DEFENSIN SYSTEM

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Staphylococcus aureus (SA), a Gram-positive pathogen, causes a wide range of skin infections from impetigo to deep abscesses. Drug-resistant strains like Methicillin-resistant *S. aureus* (MRSA), have contributed to rising infection rates, increased mortality, and growing public health concern. New therapies based on better understanding of S. aureus-host crosstalk are urgently needed. The skin's innate immune system, particularly antimicrobial peptides (AMPs) such as defensins, plays a key role in early defense against SA. Our previous work showed that defensins not only directly kill bacteria but also act indirectly by activating neutrophils through the Mrgpra2 receptor. Defensin cluster knockout (*Def* cKO) mice showed impaired immunity, with higher bacterial burden and reduced neutrophil recruitment. After entering host tissue, SA encounters environmental stress and undergoes dramatic transcriptional changes in response to host immune pressure, activating virulent genes, adjusting to nutrient limitation, and responding to immune attack. While defensins and neutrophils are known to contribute to clearance, how SA senses and adapts to their combined pressure in vivo is still unclear.
To explore this, we are comparing the transcriptomes of SA in WT, *Def* cKO, *Mrgpra2* dKO, and *S100a8*^{DTA} (neutrophil-depleted) skin to understand how SA adapts to different immune environments. Our preliminary analysis shows that, SA in mouse skin undergoes global transcriptional reprogramming within 24 hours, with increased expression of secreted virulence factors and reduced expression of surface adhesins. Compared to SA in WT skin, SA in *Mrgpra2* dKO, *S100a8*^{DTA}, and *Def* cKO showed differential expression of genes involved in cell wall remodeling, toxins, and proteases. We are testing the in vivo virulence of these key genes and comparing their loss-of-function effects in WT vs mutant animals. These findings shed light on the intricate gene regulatory network of SA, offering insights into potential therapeutic strategies against SA infections.

LEGIONELLA EMPLOY A CELL SURFACE SIGNALING SYSTEM TO MAINTAIN REPLICATION VACUOLE INTEGRITY

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The respiratory pathogen *Legionella* causes life-threatening pneumonia known as Legionnaires' disease. *Legionella* replicate in alveolar macrophages within a membrane bound compartment known as the *Legionella* containing vacuole (LCV). Maintaining LCV membrane integrity is critical for *Legionella* intracellular replication and evasion of host immune surveillance mechanisms. *Legionella* accomplish this by translocating bacterial proteins termed as effectors into the host cytoplasm that modulate host cell vesicle trafficking, ER biology and lipid metabolism. Recently, we identified a *Legionella* cell surface signaling (CSS) system that is also important for LCV stability. CSS systems function in the bacterial cell wall allowing them to monitor their environment and activate adaptive responses. Here, we show that the CSS system protects against LCV destabilization, and its critical role in this process is amplified when effectors that promote LCV integrity are deleted. This suggests a role for the CSS system in monitoring the impact of effector functions within the host cell and/or membrane integrity from within the LCV. In contrast to canonical CSS systems, the *Legionella* system appears to consist of an outer and an inner membrane protein coupled by a putative diffusible FecR domain-containing transducer protein in the periplasm, indicating a novel spatial arrangement and composition. Activation of the CSS leads to production of the second messenger cAMP that likely regulates the activity of a protease to elicit an adaptive response. While most well-characterized CSS systems function in iron/siderophore acquisition and plant-bacterium symbiosis, the identification of a CSS system employed by an intracellular pathogen to ensure LCV stability and in turn, intracellular replication, expands the role for CSS systems in bacterial pathogenesis.

ANTIGEN-SPECIFIC CD4$^+$ T CELLS PROMOTE MONOCYTE RECRUITMENT AND DIFFERENTIATION INTO GLYCOLYTIC LUNG MACROPHAGES TO CONTROL *MYCOBACTERIUM TUBERCULOSIS*.

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Although lung myeloid cells provide an intracellular niche for *Mycobacterium tuberculosis* (*Mtb*), CD4$^+$ T cells limit *Mtb* growth in these cells to protect the host. The CD4$^+$ T cell activities including interferon-γ (IFN-γ) production that account for this protection are poorly understood. Using a murine model of tuberculosis, we show that monocyte-derived macrophages (MDMs) are recruited to the lungs by *Mtb*-specific CD4$^+$ T cells via IFN-γ, which promoted the extravasation of monocyte precursors from the blood. Although the recruited MDMs were rapidly and preferentially infected, they were disinfected after receiving cognate MHCII-mediated help from CD4$^+$ T cells. MDMs receiving MHCII-mediated signals upregulated glycolytic genes associated with *Mtb* control. This activity could not be explained by IFN-γ but rather may result from the activation of CD40, whose expression by MDMs was required for immune control and whose ligand is provided by CD4$^+$ T cells during cognate interactions. Intriguingly, CD40 agonist antibody therapy was sufficient to achieve *Mtb* protection in T cell-deficient mice. Thus, *Mtb*-specific CD4$^+$ T cells attempt to clear *Mtb* from the lungs through a “catch and kill” strategy of recruiting infectable MDMs and disinfecting them through cognate MHCII-and CD40-mediated interactions. These results further suggest that macrophages, like B cells and dendritic cells, can be activated in an antigen-specific manner by cognate CD4$^+$ T cells.

MODIFICATION OF P. AERUGINOSA TRANSCRIPTION FACTOR RPON BY THE IMMUNOMETABOLITE ITACONATE PROMOTES BACTERIAL ADAPTATION TO THE AIRWAY

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Rationale: Pseudomonas aeruginosa (Pa) is a major ESKAPE pathogen associated with healthcare-related pneumonia. To adapt to the airway environment and evade host antibacterial effectors, Pa optimizes its metabolism, expression of immunostimulatory antigens, and biofilm lifestyle. The transcription factor RpoN regulates over 400 genes involved in these adaptive responses, but the host-derived signals influencing its activity are not well understood. During pneumonia the airway becomes enriched with the phagocyte-derived immunometabolite itaconate. It has an immunomodulatory role and is toxic to bacteria, yet is consumed by Pa. Itaconate modulates protein function in the host and bacteria by modifying cysteine residues. We postulate that itaconate acts as a host- derived metabolic signal for Pa, modifying the major regulatory sigma factor RpoN to drive metabolic changes which enable chronic infection.
Methods: Chemoproteomics were used to identify itaconation sites in RpoN of P. aeruginosa PAO1. Their conservation was confirmed with published sequences of clinical Pa isolates from chronic and acute infections. The roles of rpoN and conserved C218/275 residues, expected to be itaconated in vivo, were assessed in mouse pneumonia models using deletion and C218A/C275A mutants constructed in PAO1, in WT C57Bl6 and Irg1-/- mice. The differential expression of genes subject to RpoN modification by itaconate was determined by RNA-seq.The impact of itaconation on Pa metabolism was assessed by 13C metabolite labeling of bacteria
+/- itaconate.
Results: We found loss of RpoN was associated with an increased bacterial burden, inflammatory response and itaconate levels in BAL. Chemoproteomic analysis identified itaconation of cysteines at the 218 and 275 position, which were conserved in clinical isolates despite an accumulation of mutations in the rpoN gene. Constructed rpoN strains with C218A/C257A substitution, which prevent itaconation, showed reduced infectivity in WT but not in Irg1-/- mice, and reduced biofilm production as opposed to PAO1 expressing WTrpoN. Transcriptomic profiling and 13C glucose labelling revealed that cysteine availability at the 218/275 positions in the presence of itaconate enhanced glucose catabolism via the Entner-Doudoroff pathway and glucose flux to anabolic pathways.
Conclusions: RpoN responds to the phagocyte-derived metabolite itaconate and drives Pa adaptation to the lung environment. Itaconation of RpoN optimizes glucose catabolism by enhancing the Entner-Doudoroff pathways, fueling anabolic processes essential for bacterial growth.Despite frequent mutations in the rpoN gene, conservation of the itaconate-modified cysteine residues in clinical isolates suggests that modification of these sites has role in Pa adaptation during lung infection.

HUMAN NEUTROPHILS MAINTAIN AN ANTIMICROBIAL EXTRACELLULAR RNA LANDSCAPE UPON *ASPERGILLUS FUMIGATUS* CHALLENGE.

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Extracellular RNAs (exRNAs) are now recognized as potent bidirectional interkingdom effectors in plant and insect systems, but the repertoire and function of exRNA in defense against human fungal pathogens like *Aspergillus fumigatus* remains limited. Here, using small RNA-seq, we reveal a diverse neutrophil exRNA-ome capable of promoting antifungal immunity against *A. fumigatus*. Intriguingly, we observed the extracellular microRNA (miRNA) pool to be enriched for immune-regulatory and antimicrobial let-7 family sRNAs but impervious to infection status, suggesting that neutrophils produce and maintain an antimicrobial RNA environment independent of stimulus and seemingly despite well-characterized *A. fumigatus* immune repressive factors like dihydroxynaphthalene-melanin and/or gliotoxin. Cross-kingdom miRNA target prediction and exRNA ex vivo delivery experiments demonstrated that predicted putative fungal targets are modulated across kingdoms, with experiments in progress to molecularly assess putative miRNA:mRNA interactions. This regulation appeared independent of fungal argonaute proteins linked to RNA interference, but we did detect host argonautes in the extracellular fractions, suggesting host RNA binding proteins may contribute to interkingdom cargo delivery. Unlike the miRNAs, extracellular tRNA fragments (tRFs) did display some alterations in response to infection, possibly providing an additional arm to antifungal immunity and offering potential as biomarkers for detection of fungal infection. Optimization of a droplet digital stem-loop RT-PCR detection protocol for the tRFs offers a first step in this direction, while future investigations will be required to assess the influence of the tRFs on host defense and immune coordination. In conclusion, our study provides an improved understanding of the complex host extracellular RNA landscape in response to a deadly human fungal pathogen.

DEFINING MACROPHAGE-INDUCED STRESSORS OF *BACILLUS ANTHRACIS* AT EARLY STAGES OF ANTHRAX DISEASE

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Bacillus anthracis, the causative agent of anthrax, is a Gram-positive, spore-forming bacterial pathogen known for its bioterror potential. During inhalation anthrax, the most lethal form of the disease, inhaled spores are phagocytosed by macrophages in which they germinate, persist, and disseminate throughout the body. The stressors encountered within these macrophages, and the mechanisms by which the bacterium resists these stressors, remain incompletely understood. A better understanding of these early host-pathogen interactions may reveal targets for novel therapeutics to treat anthrax. Therefore, the objective of this study is to comprehensively identify host genes that contribute to the cell envelope stress and iron starvation experienced by *B. anthracis* within macrophages. This study will also identify host genes that impact the bacterium's ability to germinate and persist within macrophages. To accomplish this, fluorescent *B. anthracis* strains were engineered to express mScarlet-I3 constitutively, and express mNeonGreen driven by promoters that are responsive to cell envelope stress or iron starvation. Using a robotic liquid handler, a comprehensive arrayed library of CRISPR guides will be used to inactivate murine macrophage genes in microwell plates. These mutant macrophages will be infected with the fluorescent reporter bacteria, and high-content imaging will be used to assess the impact of the gene knockout on bacterial germination, growth, and stress signaling. A similar comprehensive screen has been successfully employed to identify genes which impact the growth and stress signaling of *Staphylococcus aureus* in macrophages. Preliminary work has validated that the experimental setup can visualize *B. anthracis*-macrophage interactions in a high throughput manner. Additionally, we have used our fluorescent strains to visualize *B. anthracis in vivo* using multi-photon microscopy on organs from infected mice. Once completed, this study will yield a comprehensive molecular atlas of host genes that impact the growth of *B. anthracis* in macrophages. Further studies will elucidate the impact of these interactions on anthrax pathophysiology.

IRF2 DEGRADATION TUNES THE INNATE IMMUNE RESPONSE

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The transcription factor IRF2 protects against skin inflammation in mice and humans but, paradoxically, promotes pyroptosis by inducing Gsdmd. How IRF2 activates some proinflammatory genes, but suppresses others is unclear. We show that skin inflammation in Irf2-deficient mice is driven by IRF1 activation of interferon-stimulated genes (ISGs). Chromatin profiling revealed that IRF1 and IRF2 occupy the same ISG regulatory sites, but as a weaker transcriptional activator, IRF2 limited ISG transcription by IRF1. Toll-like receptor signaling favored IRF1-driven transcription by inducing Irf1. In addition, IRF1 recruited the ubiquitin ligase SPOP to ISG sites, resulting in proteasomal degradation of IRF2. This shift from IRF2 to IRF1 occupancy enhanced ISG transcription. Collectively, these findings define a hierarchical transcriptional circuit in which IRF2 limits IRF1 activity under homeostatic conditions but is displaced during an immune response, allowing IRF1-dependent gene programs central to innate immunity and autoinflammation.

THE INTRINSICALLY DISORDERED REGION OF THE *LISTERIA MONOCYTOGENES* SECRETION CHAPERONE PRSA2 IS CRITICAL FOR BACTERIAL VIRULENCE AND CLIENT INTERACTIONS

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This study characterizes the intrinsically disordered region of the secreted Gram-positive chaperone PrsA2 in *Listeria monocytogenes* (*Lm*). While 30-40% of eukaryotic proteins contain at least one intrinsically disordered region (IDR), only 4% of bacterial proteins contain IDRs, therefore investigating these disordered regions is critical for understanding protein function in bacteria. Here, we use the food-borne pathogen *Lm* to characterize of an IDR within a critical peptidyl-prolyl isomerase (PPIase) chaperone, PrsA2. Highlighting some of the most important aspects of this work, we demonstrate that the PrsA2 C-tail IDR is critical for *Lm* virulence in the mouse septicemic model and for the function (interaction and folding) of the major virulence factor and pore forming toxin, listeriolysin O (LLO). Moreover, the PrsA2 C-tail is necessary for overcoming cell-wall targeting antibiotics suggesting interactions with additional key factors. Further, since the intrinsically disordered nature of this C-tail IDR is conserved in many PrsA homologs; our results may be widely applicable to other important Gram-positive pathogens and adds to the newly expanding characterization of IDRs within bacterial proteomes.

THE CONTRIBUTION OF *SERRATIA* NUCLEASE IN THE GENERATION OF DEOXYNUCLEOTIDES VIA THE DEGRADATION OF NEUTROPHIL EXTRACELLULAR TRAPS

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Serratia marcescens is a Gram-negative opportunistic pathogen commonly associated with nosocomial infections. Treatment of infections caused by *S. marcescens* is often complicated by multidrug resistance. Neutrophils play a crucial role in the control of *Serratia* infection and individuals with chronic granulomatous disease are prone to infections caused by this organism. Neutrophils facilitate pathogen clearance by phagocytosis and the release of Neutrophil Extracellular Traps (NETs), fibrous structures composed of decondensed chromatin and decorated with around 30 different proteins including neutrophil elastase, myeloperoxidase, and cathelicidin. Some bacteria use extracellular nucleases to degrade the DNA component of NETs and avoid entrapment. Furthermore, certain Gram-positive bacteria have a surface anchored 5'-nucleotidase (AdsA of *Staphylococcus aureus*) which can convert nuclease-digested NET components into deoxyadenosine (dAdo), a chemical which triggers noninflammatory apoptosis in macrophages. The production of dAdo can be monitored through quantification of orthophosphate, a byproduct of the 5'-nucleotidase mediated hydrolytic dephosphorylation of deoxyadenosine monophosphate (dAMP). *S. marcescens* is known to produce a non-specific extracellular nuclease (NucA); however, the role of NucA in NET degradation has not been previously described. Additionally, *S. marcescens* encodes a periplasmic protein UshA (TBU70554) with 28% identity and 42% similarity to the amino acid sequence of *S. aureus* AdsA 5'-nucleotidase. Therefore, UshA is a candidate enzyme for the conversion of degraded NETs into dAdo. Incubation of bacterial lysate or washed wild type *S. marcescens* cells with dAMP resulted in orthophosphate release, indicating 5'-nucleotidase activity.

Here, we show that HL-60 cells differentiated into neutrophil-like cells (dHL-60) can be used to study the role of *S. marcescens* enzymes in NET evasion *in vitro*. dHL-60 cells generate NETs in response to chemical stimulation by PMA (phorbol 12- myristate 13-acetate) and to *S. marcescens* exposure. Wild type *S. marcescens* degraded and escaped from NETs while nuclease-deficient Δ*nucA* were trapped and killed. Incubation of *S. marcescens* with cell-free dHL-60 NETs resulted in orthophosphate release in wild type but not Δ*nucA*. Complementation restored the ability of Δ*nucA* to escape from NETs and generate orthophosphate when exposed to NETs. These results support our hypothesis that the degradation of NETs by nuclease is necessary for the downstream generation of deoxynucleotides and orthophosphates. Efforts to create Δ*ushA* and Δ*nucA*Δ*ushA* mutant strains are currently underway.

HELICOBACTER PYLORI VACA ALTERS CHOLESTEROL HOMEOSTASIS IN HUMAN GASTRIC EPITHELIAL CELLS.

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Helicobacter pylori is a bacterium that can establish long-term colonization of the human stomach. *H. pylori* strains harboring certain virulence factors, including the active form of the pore-forming toxin vacuolating cytotoxin A (VacA), can promote the development of peptic ulcer disease and gastric cancer. Although VacA intoxication elicits multiple alterations in gastric cells, the mechanisms by which VacA promotes *H. pylori* fitness and modulates host cell function remain incompletely understood. In this study, we analyzed the transcriptome of a gastric epithelial cell line (AGS) treated with purified VacA. Differential expression analysis revealed cholesterol biosynthesis as the most significantly upregulated pathway in VacA-treated cells. RT-qPCR analyses confirmed these results and yielded similar findings in another gastric epithelial cell line (MKN-28) and primary human gastric epithelial cells. Treatment of AGS cells with channel-deficient VacA mutant proteins did not result in increased transcript abundance of cholesterol biosynthesis genes, indicating that VacA channel activity is required for these alterations. Co-culture of AGS cells with *H. pylori* strains producing VacA stimulated increased transcript abundance of cholesterol biosynthetic genes compared to co-culture with isogenic vacA null mutant strains. We utilized LC-MS/MS to analyze the abundance of sterols in AGS cells treated with VacA or control buffer. We found that VacA causes non-uniform alterations in cellular sterol concentrations, with proximal pathway sterols increasing in abundance in response to VacA and some distal pathway sterols decreasing. There were no significant effects of VacA on cellular cholesterol concentrations in AGS cells cultured in lipid-depleted media, but VacA-treated cells cultured in lipid-replete medium had lower cholesterol levels than buffer-treated cells. Supernatants from VacA-treated AGS cells cultured in lipid-depleted media contained higher levels of cholesterol than supernatants from control-treated cells. These results suggest that VacA treatment leads to an efflux of cholesterol from cells. This efflux may stimulate the activation of cholesterol biosynthesis gene expression to compensate for losses in cellular cholesterol. We speculate that VacA-induced alterations in cholesterol homeostasis may be relevant for cholesterol acquisition by *H. pylori*, a cholesterol auxotroph, during long-term colonization of the stomach.

STAPHYLOCOCCUS AUREUS A-TOXIN IMPAIRS THE ANTIGEN-SPECIFIC CD4$^+$ T CELL RESPONSE IN AN ADAM10-DEPENDENT MANNER

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Staphylococcus aureus is a leading cause of global mortality from bacterial infections, accounting for over 1 million deaths in 2019 and an annual economic burden exceeding $10 billion in the United States alone. Despite advances in antimicrobial therapy, mortality from severe *S. aureus* infections has plateaued at roughly 20%, and vaccine development has remained largely unsuccessful. Understanding the molecular and cellular pathways by which *S. aureus* alters host immunity is critical for guiding rational vaccine design. In a cohort of pediatric patients, we observed that primary skin infections were associated with nearly 50% rates of recurrent S. aureus disease, whereas invasive infections typically conferred protection, suggesting that the site of infection influences host immunity. Murine studies revealed that skin infection with *S. aureus* impairs the development of antigen-specific CD4$^+$ T cell immunity via the action of α-toxin (Hla), a pore-forming toxin. Notably, Hla exposure exclusively in the context of skin infection causes loss of antigen-presenting cells and depletion of antigen-specific CD4$^+$ T effector and memory compartments. In prior work, we identified ADAM10 as the eukaryotic cellular receptor for Hla and demonstrated its essential role across *S. aureus* disease states, including sepsis, pneumonia, and skin infections. Both active and passive immunization targeting Hla conferred protection across these clinical presentations, highlighting Hla as a consensus vaccine target. Yet, the precise mechanism by which Hla impairs antigen-specific CD4$^+$ T cell responses has remained undefined. We hypothesize that Hla causes a global, ADAM10-dependent functional defect across the CD4$^+$ T cell population, representing a critical barrier to effective vaccination. In this study, we demonstrate key advances addressing this knowledge gap. By flow cytometry, histology, and ELISA, we found that Hla reaches the skin-draining lymph node (dLN) following subcutaneous infection. In both in vivo and ex vivo studies, we demonstrate that Hla exposure alters the proliferation, differentiation, and cytokine profile of CD4$^+$ T effector and memory compartments. We provide mechanistic insight on how the specific attributes of Hla as a pore-forming cytotoxin directly perturb T cell signal transduction in an ADAM10-dependent manner, extending these studies to reveal a direct correlation between human ADAM10 expression on the CD4+ T cell and toxin-mediated impairment of cellular signaling. These findings, which span both murine and human systems, suggest that Hla acts within the dLN to broadly disable the host's ability to mount effective T cell–mediated immunity. A deeper understanding of this pathway will have far-reaching implications for vaccine design and implementation, guiding the development of Hla-neutralizing strategies early in life to protect the T cell compartment.

INVESTIGATING THE ROLE OF BHLHE40 IN MODULATING HOST IMMUNE RESPONSES DURING *MYCOBACTERIUM TUBERCULOSIS* INFECTION

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Mycobacterium tuberculosis (*Mtb*), the causative agent of tuberculosis, remains a global health threat, with the World Health Organization reporting 1.25 million deaths in 2023. While the immune factors required to control *Mtb* pathogenesis remain incompletely defined, we have previously demonstrated that the host transcription factor BHLHE40 is essential for controlling *Mtb* infection. Mice deficient in *Bhlhe40* (*Bhlhe40*$^{-/-}$) are highly susceptible to *Mtb* infection, with most succumbing to infection by 30 days post infection (dpi). Further studies revealed that BHLHE40 is required in T cells and CD11c+ lung macrophages and dendritic cells (DCs) to regulate inflammatory responses and limit *Mtb* replication, in part by repressing *Il10* expression. Additionally, elevated IL-10 levels that occur in *Bhlhe40*$^{-/-}$ mice partially contribute to *Mtb* susceptibility; however, how IL-10 impairs the control of *Mtb* remains unclear. To determine how excess IL-10 impacts immune responses during *Mtb* infection, we used flow cytometry to examine lung cell populations in *Mtb*-infected wildtype (WT), *Bhlhe40*$^{-/-}$, and *Bhlhe40*$^{-/-}$ mice deficient in *Il10*. Lung-resident DCs, monocyte-derived DCs, and T cells (CD4+, CD8+) were significantly reduced in *Bhlhe40*$^{-/-}$ mice compared to WT mice, suggesting that BHLHE40 may regulate the recruitment and survival of these cells. Notably, the deletion of *Il10* in *Bhlhe40*$^{-/-}$ mice restored these cell populations to WT levels, indicating that the IL-10 produced in the absence of *Bhlhe40* reduces DC and T cell populations and may hinder adaptive immune responses during *Mtb* infection. To determine which immune cell responses to elevated IL-10 contribute to the susceptibility of *Bhlhe40*$^{-/-}$ mice, we assessed the survival of *Mtb*-infected *Bhlhe40*$^{-/-}$ mice with cell-specific deletions of the IL-10 receptor (*Il10rα*) in distinct immune populations. We found that *Bhlhe40*$^{-/-}$*Il10rα*$^{fl/fl}$*-LysM-Cre* and *Bhlhe40*$^{-/-}$*Il10rα*$^{fl/fl}$*-CD11-Cre* mice had median survivals of 33 and 36 dpi, respectively, with ~10% surviving past 80 dpi, indicating that IL-10 signaling in LysM+ and CD11c+ cells partially contributes to *Bhlhe40*$^{-/-}$ mouse susceptibility. However, the survival of these mice did not recapitulate the survival of *Bhlhe40*$^{-/-}$*Il10rα*$^{-/-}$ *mice* (104 dpi), suggesting that susceptibility might be due to a combined effect of IL-10 signaling in both LysM+ and CD11c+ cells. To test this, we monitored the survival of *Bhlhe40*$^{-/-}$*Il10rα*$^{fl/fl}$*-LysM-Cre-CD11c-Cre* mice and observed a median survival of 97 dpi, which is comparable to that of *Bhlhe40*$^{-/-}$*Il10rα*$^{-/-}$ mice. These findings demonstrate that IL-10 signaling in both LysM+ and CD11c+ cells contributes to *Bhlhe40*$^{-/-}$ mouse susceptibility. Future studies will examine how IL-10 dysregulation in *Bhlhe40*$^{-/-}$ mice alters adaptive immune responses during *Mtb* infection.

MRSA ESCAPES PATHOGEN SENSING BY HUMAN MONOCYTES VIA TOXIN-MEDIATED MYDDOSOME DISRUPTION

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Introduction: Methicillin-resistant Staphylococcus aureus (MRSA) is one of the leading pathogens causing severe bloodstream infections like sepsis. MRSA infection suppresses immune sensing in human blood. Here, we show that MRSA targets human monocytes to blunt cytokine production, an immunosuppression mediated by the pore-forming leukocidins, particularly LukAB. Mechanistically, LukAB pores were found to disrupt the formation of the Myd88, IRAK4 and TRAF6 myddosome complex, consequently preventing MAPK (mitogen-activated protein kinase) activation and transcription of cytokine genes.
Methods: Wild-type (WT) community-acquired MRSA strain (USA300), USA300 deficient in bicomponent pore-forming toxins (*Δtoxins*) or lukAB (*ΔlukAB*) were cultured in tryptic soy broth overnight followed by subculture for 3h at 37˚C. Peripheral blood mononuclear cells (PBMCs) were isolated from human blood and monocytes purified using human CD14 microbeads. For infections, $1X10^6$ monocytes were challenged with a multiplicity of infection (MOI) of two for 30min to 2h at 37˚C. Alternatively, monocytes were treated with 1-2ng/mL of LukAB ± 1μg/mL LPS (lipopolysaccharide) to study TLR (Toll-like receptor) activation. After treatments, the monocytes were isolated by centrifugation and used to access MAPK activation and myddosome assembly, and the supernatants were used to measure cytokines.
Results: WT USA300 blunted the production of cytokines/chemokines (e.g. IL-8, MCP1, MIG, MIP-1α, MIP-1β, TNFα and VEGFA) when compared to cells infected with *Δtoxins* or *ΔlukAB*. Genetic complementation or the addition of purified WT-LukAB but not an oligomerization deficient LukAB reduced cytokine production by *Δtoxins* or *ΔlukAB*. WT USA300 reduced the phosphorylation of p38, JNK and MAPK proteins. Using expansion microscopy, we show that Myd88 and IRAK4 assembly is disrupted upon WT-USA300 infection. Pre-treatment with LPS (a potent TLR4 activator) relieved LukAB-mediated suppression of MAPK activation and cytokine production.
Conclusion: MRSA infection leads to the inhibition of cytokine response by primary human monocytes by interfering with the formation of the TLR-myddosome complex via the pore-forming toxin LukAB. Thus, we describe herein a novel immunosuppressive strategy by MRSA.

This project was funded by NIH-NIAID 02R01 AI099394-10

EXPLORING THE INFLUENCE OF PROPOFOL ON INNATE IMMUNITY AND *KLEBSIELLA PNEUMONIAE* PATHOGENESIS

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Hospital-acquired infections (HAIs), including surgical site infections (SSIs), remain an ongoing problem in the United States, dramatically increasing healthcare costs through longer stays, higher readmission rates, and additional treatment costs. The estimated annual cost of SSIs in the US is between $3.3 billion and $10 billion. Propofol is a commonly used anesthesia induction agent in hospitals before surgery, in the ICU, and in routine procedures such as colonoscopies. It is valued for its rapid onset and offset of anesthesia and its safety profile when administered by trained professionals. Propofol induces anesthesia by potentiating GABA-A receptor-mediated inhibition of neural activity in the brain. Previous research has shown that propofol exacerbates bloodstream infections caused by *Listeria monocytogenes* and *Staphylococcus aureus*. Given that patients who are intubated in the ICU are often placed on propofol for prolonged periods, we have investigated a mouse model of respiratory infection using the bacterial pathogen *Klebsiella pneumoniae (Kp)*. Using a transposon-based Tn-seq approach and screening mutants of hypervirulent *Kp* KPPR1, we identified several virulence factors critical for infection in the context of propofol versus ketamine anesthesia, including the *mlaC* gene. Genes of the *mla* pathway have been shown to regulate phospholipid transport in *Escherichia coli*. We found that using a low inoculum dose of 200 CFU, *Kp mlaC* deletion mutants exhibited significantly reduced bacterial loads in propofol-treated mice compared to ketamine/xylazine sedated controls, a phenotype that appears to correlate with increased sensitivity of *mlaC* mutants to antimicrobial peptides. Preliminary data suggest that propofol may enhance host antimicrobial peptide production, and thus, we propose that a propofol-dependent increase in host antimicrobial peptides enhances the clearance of *Kp mlaC* within the lungs of infected mice. *mlaC* deletion mutants retain capsule expression and are serum resistant; however, preliminary data suggest that capsule modifications may be present. Overall, analyses of the outcome of infection with specific *Kp* mutant strains are helping us to better understand the impact of propofol sedation on host immune function.

FATTY ACID METABOLISM AFFECTS *STAPHYLOCOCCUS AUREUS* HEME HOMEOSTASIS

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Staphylococcus aureus is a leading cause of global morbidity and mortality. *S. aureus* requires the acquisition of heme to colonize its host and cause disease. While heme is essential for numerous biochemical functions, including energy generation and detoxification of environmental stresses, accumulation of excess heme within cells causes toxicity. Heme toxicity can occur via the induction of reactive oxygen and nitrogen species, or via disrupting cellular membrane and compromising membrane integrity and functions. Thus, the balance between heme utility and toxicity is tightly regulated. Through a transposon-sequencing (Tn-seq) approach, we identified genes in the cell membrane biogenesis and lipid metabolism pathways that are conditionally essential for *S. aureus* growth in the presence of heme. Many of these identified genes are involved in assimilation of exogenous fatty acids (ExoFAs). To determine the contribution of ExoFAs to *S. aureus* heme homeostasis, we subjected *S. aureus* to toxic levels of exogenous heme, in addition to sublethal levels of ExoFAs. We observed that growth of wildtype (WT) S. aureus is rescued with the addition of ExoFAs regardless of chain length and saturation. A strain inactivated for *fakA*, a gene encoding the fatty acid kinase involved in incorporation of ExoFAs into the cell membrane, has a growth advantage over WT under heme toxicity. This suggests a role for free fatty acids in heme detoxification. In parallel, expression of *plsX*, encoding for acyltransferase of phospholipid biosynthesis intermediates, is downregulated in the presence of toxic levels of heme, supporting the hypothesis that ExoFAs are not converted into phospholipids, but instead remain as free fatty acids to mediate heme stress. Taken together, these results establish a relation between heme homeostasis and fatty acid metabolism in *S. aureus*. The findings of this work lay ground for further investigation of the contribution of ExoFAs to *S. aureus* survival and pathogenesis.

FTY720, A SPHINGOSINE 1-PHOSPHATE RECEPTOR MODULATOR, AMELIORATES EXPERIMENTAL COLITIS BY MODULATING AKKERMANSIA MUCINIPHILA ABUNDANCE AND THE STAT3/TH17 AXIS

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The targeting of sphingosine-1-phosphate (S1P) signaling is an emerging therapeutic strategy for immune-mediated diseases, including inflammatory bowel disease (IBD). Although gut microbiota play a crucial role in the pathogenesis of IBD, little information is available on how microbiota are changed by S1P receptor modulators.
We investigated the effects of FTY720 on the immune system and gut microbiota in mice with colitis induced by dextran sulfate sodium (DSS). FTY720 prevented weight loss and decreased levels of inflammatory cytokines (tumor necrosis factor-α, IL-1β, IL-6, and IL-17) and fibrotic markers (α-smooth muscle actin, collagen type I, and transforming growth factor-β) in the colon. Expression of Th17 and Treg cells increased in *ex vivo* spleens and mesenteric lymph nodes of FTY720-treated mice with DSS-induced colitis. However, FTY720 inhibited IL-17 expression in mouse CD4+ T cells and peripheral blood mononuclear cells of patients with ulcerative colitis (UC) *in vitro* by regulating signal transducer and activator of transcription 3. The composition of the gut microbiome altered significantly in FTY720-treated mice. The *Firmicutes:Bacteroidetes* ratio and *Akkermansia muciniphila* significantly increased in FTY720-treated mice. The gut microbiota of patients with UC was different from that of healthy controls. The *Firmicutes:Bacteroidetes* ratio and *A. muciniphila* decreased significantly in patients with UC.

Conclusions: These data suggest that FTY720 has a therapeutic effect on IBD by regulating pro-inflammatory signals and restoring the profile of gut microbiota.

PROTEOMIC INSIGHTS INTO PNEUMOCOCCAL ADAPTATION AND COMPETENCE ACTIVATION DURING PNEUMONIA-DERIVED SEPSIS

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Streptococcus pneumoniae (pneumococcus) is a major cause of pneumonia, pneumonia-derived sepsis (pneumonic sepsis), multi-organ dysfunction, otitis media, and meningitis. Limited serotype coverage in current vaccines and increased antibiotic resistance have hampered the eradication of pneumococcal diseases. The pneumococcal competence system is long known to be required for genetic transformation, which develops naturally in response to the threshold accumulation of the peptide pheromone competence stimulating peptide (CSP). The short burst of competent state (~40 minutes) augments the expression of three distinct sets of "early", "late", and "delayed" competence (*com*) genes *in vitro*. However, in contrast to a short burst of competent state *in vitro*, our recent IVIS-based imaging studies revealed that during acute pneumonic sepsis in mice, multiple pneumococci strains can only enter the competent state approximately 24 hours after lung infection. This is immediately followed by a breach of the alveolar-capillary barrier and invasion into the bloodstream. The competent state during pneumonic sepsis is prolonged and persistent, lasting for >30 hours until animals become moribund. Our RNA-Seq reveals that 20%, 42%, and 24% of the top 50 most upregulated pneumococcal genes in infected mouse lungs at 12-hpi, 24-hpi, and >40-hpi are the *com* genes, suggesting crucial roles played by the competence regulon in the initial adaptation to the lung microenvironment, followed by lung infection and breaching of the alveolar-capillary barrier, and maintenance of the septicemic stage. Despite the aforementioned progress, the underlying factors and mechanisms regulating pneumococcal adaptation to the lung environment conducive to entrance into the competent state remain unknown. We hypothesize that initial disruption of the alveolar-capillary leaks lung-restricted host factors and metabolites from blood, permitting the pneumococcus to enter the competent state. To elucidate these processes, we are now employing a proteomics approach to define the molecular pathways linking barrier breakdown to sustained competence activation, aiming to identify novel therapeutic targets against invasive pneumococcal disease.

GENOME-WIDE ASSOCIATION ANALYSIS REVEALS GENETIC FEATURES UNDERLYING SITE-SPECIFIC ADAPTATION IN CLINICAL ISOLATES OF PSEUDOMONAS AERUGINOSA

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The Gram-negative opportunistic bacterium Pseudomonas aeruginosa causes a wide range of infections, including bloodstream and chronic respiratory infections in individuals with structural lung diseases such as cystic fibrosis (CF). Extensive genomic diversity has been previously observed in P. aeruginosa populations inhabiting distinct environments in the human host. However, the specific genetic determinants underlying adaptation to different infection sites remain poorly understood. In this study, we performed a genome-wide association study (GWAS) on 1,089 publicly available P. aeruginosa genomes, comprising 249 from bloodstream infections and 840 from sputum samples of CF patients, to identify genetic variants associated with infection site-specific adaptation. Using pan-genome analysis, we identified 69,129 genes in the dataset, including 3,597 core genes. We analyzed 118,230 single nucleotide polymorphisms (SNPs), 296,967 unitigs, and 1,983 accessory genes using linear mixed models that accounted for population structure. A total of 298 SNPs, 177 unitigs, and 11 accessory genes were significantly associated with infection site. Among the significant SNPs, genes enriched in bloodstream samples were involved in drug efflux, iron metabolism, nutrient acquisition, and oxidative stress response, whereas the only sputum-enriched gene was amrZ, a regulator of motility and biofilm formation. Accessory gene GWAS revealed further site-specific enrichment. In bloodstream isolates, pstA1, a high-affinity phosphate transporter gene critical for survival in nutrient-limited conditions, was enriched. Sputum isolates showed enrichment for oprB_2, imuB, and zwf_1, genes involved in carbohydrate uptake, stress-induced mutagenesis, and oxidative stress defense, respectively. These results suggest functional adaptation to nutrient scarcity in the bloodstream and to fluctuating metabolic and immune challenges in the respiratory tract. Analysis of 110 matched isolate pairs confirmed these findings, revealing consistent enrichment of phenazine biosynthesis genes (e.g., phzA2, phzB1) in sputum and cytotoxicity-related genes (e.g., cdiA) in blood. We also estimated narrow-sense heritability to assess the contribution of each genetic feature to phenotypic variation. SNPs represented the highest proportion (73.7%), followed by accessory genes (61.2%) and unitigs (25.0%). Overall, our findings offer important insights into the genetic mechanisms that support P. aeruginosa's success as an opportunistic pathogen and its ability to adapt to distinct niches in the human host.

A RODENT MODEL OF SEVERE INJURY TO INVESTIGATE TRAUMA-ASSOCIATED FUNGAL INFECTION

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Polytraumatic injuries, severe injuries to two or more areas of the body, drive a progressively dysregulated systemic immune response marked by both hyperinflammation and prolonged immunosuppression. These injuries increase susceptibility to infections, potentially leading to devastating complications that include delayed wound healing, amputation, sepsis, and mortality. Invasive fungal infections (IFIs) are a particular area of growing concern among trauma-associated infections. They largely originate from wound contamination with environmental molds, such as zygomycetes, *Aspergillus*, and *Fusarium*and are characterized by angioinvasion and rapid dissemination. Importantly, trauma-associated IFIs are frequently associated with antifungal resistance and co-infection with other multidrug-resistant organisms, highlighting an urgent need for alternative therapeutic strategies. Immune checkpoint inhibitors (ICIs), originally developed for cancer, have recently shown promise in treating diseases underlined by immunosuppression. Immune checkpoint proteins, such as PD-1, are expressed on activated T and B cells, NK cells, and mononuclear phagocytes, where they regulate the strength and duration of immune responses, limiting inflammation and self-recognition. Recently, ICIs have shown promise in the treatment setting of sepsis, with a number of preclinical studies, case studies, and phase 1b clinical trials reporting favorable outcomes. However, whether ICIs can mitigate polytrauma-associated infections has not been explored.

We developed a novel rodent model of polytrauma-associated fungal infection by combining a severe burn with a muscle cryoinjury on an unburned extremity, which is contaminated with the pathogenic mold, *Mucor circinelloides*. Compared to animals with muscle injury infection alone, polytrauma-injured infected animals display an increase in fungal invasion and delayed recovery rates. Polytrauma-injured mice also experience a systemic immunosuppressive response, as evidenced by a decrease in circulating T cells and an increase in the immunosuppressive markers PD-1, Lag-3, and Tim-3, akin to that observed in human trauma patients. Notably, treatment with anti-PD-1 significantly increased weight gain and circulating T cell numbers compared to controls. Ongoing work is examining the downstream effects of anti-PD-1 therapy on fungal pathogenesis, sepsis, wound healing, and immune recovery. By establishing a novel preclinical mouse model for polytrauma-associated infection, our work represents an important step in advancing therapeutic care in trauma patients. While current efforts focus on the fungal pathogen *Mucor circinelloides*, delineating the impact of immune checkpoint inhibitor treatment in polytrauma-infections has broad relevance for both fungal and bacterial infections.

THE *MYCOBACTERIUM TUBERCULOSIS* SECRETED PROTEIN RV1075C MANIPULATES HOST HISTONE METHYLTRANSFERASES TO PROMOTE INFECTION

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Mycobacterium tuberculosis (Mtb) is one of the most infectious and deadly pathogens in the world. Key to Mtb virulence are Mtb membrane-bound and secreted effector proteins that manipulate the host-pathogen interface to promote Mtb survival. Earlier studies identified roughly 100 putative effector proteins that can be secreted by Mtb, but the molecular mechanisms through which these proteins promote Mtb pathogenesis remain elusive. A growing body of literature suggests that many intracellular bacterial pathogens secrete a class of effector proteins—termed nucleomodulins—that traffic to the host cell nucleus and target complexes involved in chromatin remodeling, histone modification, transcription, and pre-mRNA splicing. An *in silico* screen identified a putative nuclear localization signal in the Mtb secreted protein Rv1075c, and ectopic expression showed that Rv1075c localized to the macrophage nucleus and biochemically associated with chromatin. Subsequent IP/MS experiments identified interactions between Rv1075c and multiple members of the SET1 histone methyltransferase complex (ASH2L, WDR5, RBBP5, DPY30), which deposits H3K4me3 and is generally associated with active transcription. To begin to implicate Rv1075c in Mtb pathogenesis, we generated a ΔRv1075c Erdman strain of Mtb. At early time points post-infection, ΔRv1075c Mtb elicits less *Ifnb1* expression than a WT control, and ectopic expression of Rv1075c potentiates *Ifnb1* and interferon-stimulated gene expression in cytosolic DNA-stimulated macrophages. Additional CUT&RUN data indicate that there are broad changes in chromatin remodeling and innate immune gene expression in the presence of ectopically expressed Rv1075c, thus highlighting a novel and unappreciated role of Rv1075c in changing chromatin accessibility. We have also found that mice infected with ΔRv1075c had lower transcript levels of *Set1A*, an enzyme associated with the SET1 methyltransferase complex, which indicates that Rv1075c is influencing SET1 methyltransferase activity. Together, our data suggest that Mtb secretes Rv1075c to manipulate the host-pathogen interface at the level of the SET1 histone modifying complex to tune cytosolic DNA sensing and induce a pro-bacterial type I IFN response.

SMALL MOLECULES SECRETED BY *PSEUDOMONAS AERUGINOSA* KILL *ACANTHAMOEBA CASTELLANII*

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Acanthamoeba castellanii is a pathogenic free-living amoeba (FLA) that causes fatal central nervous system infections in immunocompromised hosts, as well as sight-threatening amebic keratitis (AK) in healthy contact lens wearers. *Pseudomonas aeruginosa* are ubiquitous environmental Gram-negative bacteria that co-exist with *A. castellanii* and are prey for these bacteriovorous FLAs. Given the strong evolutionary pressure placed on *P. aeruginosa* by *A. castellanii*, we hypothesized that *P. aeruginosa* secretes amoebicidal compounds to defend itself against amoebal grazing. Using several orthogonal assays to measure trophozoite and cyst viability, we demonstrated that the cell-free supernatants of several *P. aeruginosa* strains are lethal to *A. castellanii* (Neff) and that <3 kDa small molecules are specifically responsible for trophocidal activity. To identify genes required for amoebicidal activity, we individually screened supernatants prepared from an arrayed PA14 transposon library and identified a hit in FabY, a non-canonical ketosynthase which initiates fatty acid synthesis in *P. aeruginosa*. FabY activity influences the production of rhamnolipids, quinolones, and phenazines; these secondary metabolites have been broadly implicated in inter-kingdom signaling and toxicity. Mutants disrupting enzymes in rhamnolipid, quinolone, and phenazine biosynthetic pathways were constructed and tested (singly and in combination) to identify FabY-dependent amoebicidal molecules. Although PA14 Δ*fabY* supernatant is severely attenuated for trophozoite killing, PA14 Δ*phzA1-G1*Δ*phzA2-G2*Δ*rhlR*Δ*rhlI*Δ*pqsA* supernatant retains trophocidal activity. Efforts are currently underway to identify metabolites differentially present in active vs. inactive supernatants, using MS and NMR, while a bioassay-guided fractionation pipeline is being used to identify active molecules in the PA14 Δ*phzA1-G1*Δ*phzA2-G2*Δ*rhlR*Δ*rhlI*Δ*pqsA* supernatant.
Through these complementary approaches we hope to identify and understand how compounds secreted by *P. aeruginosa* are lethal to *A. castellanii*. Knowledge of such natural compounds and their mechanism of action both illuminates ecological predator-prey interactions and may lead to the discovery of natural products with important clinical indications.

THE PUTATIVE *BORRELIA HERMSII* GLUTATHIONE PEROXIDASE IS REQUIRED FOR SURVIVAL DURING ROS AND RNS CHALLENGES

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Tick-borne relapsing fever (TBRF) is caused by bites from ticks carrying relapsing fever species of *Borrelia* (*B. hermsii* and *B. turicatae* in the United States). TBRF causes recurrent high fevers corresponding to relapses and resolutions of spirochetemia. This contrasts with Lyme disease (LD) *Borrelia* (*B. burgdorferi*) which are carried by hard ticks and can cause symptoms such as erythema migrans, arthritis, carditis, or neuropathy. TBRF *Borrelia* are understudied, resulting in a paucity of knowledge of these spirochetes' physiological systems. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are products of oxidative metabolism and critical components of antimicrobial host responses. In the mammalian host, ROS/RNS is produced by immune cells to eliminate pathogens. Additionally, *Ornithodoros turicatae* (the soft tick vector for *B. turicatae*) produces ROS in salivary glands and midgut, sites where these spirochetes must persist prior to transmission. In all organisms, glutathione peroxidases (Gpx) function to reduce hydroperoxides and peroxynitrites using glutathione to protect cells from ROS/RNS. Gpx homologs are only found in the soft tick RF species *B. hermsii* (*Bh*) and two hard tick transmitted RF *Borrelia*, making *Bh* unique among soft tick RF species and LD species. Thus, we hypothesized that *bh0519A*, an annotated Gpx, is important for Bh survival in its tick vector and/or mammalian host and generated a *bh0519A* mutant (*Δbh0519A*) by allelic exchange. Our data show *Δbh0519A* has reduced growth in a microaerobic environment, which was further exacerbated in the absence of sodium pyruvate (ROS scavenger). Using hydrogen peroxide (ROS) and DEA/NO (RNS) challenges, we show *bh0519A* is required for *Bh* protection in vitro. Surprisingly, *bh0519A* is dispensable for relapsing *Bh* spirochetemia in the murine infection model but may be required for competition against wild type *Bh* in a competitive infection. We will assess the contribution of *bh0519A* in *Bh* survival within in its tick vector and ex vivo in the presence of activated murine macrophages and neutrophils. We will also determine whether *bh0519A* is required to prevent DNA damage due to ROS damage. Using mass spectrometry, we will determine whether RNS-injured proteins (S-nitrosylated/nitrotyrosinated proteins) are produced due to lack of *bh0519A*. We will also confirm the Gpx activity of recombinant BH0519A. Together, these studies characterize a putative Gpx in *Bh* and evaluate how the spirochete copes with ROS/RNS within the enzootic cycle.

INTERACTIVE EFFECTS OF MICROPLASTIC EXPOSURE ON STREPTOCOCCUS PNEUMONIAE INFECTION AND VIRULENCE

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Streptococcus pneumoniae is a gram-positive diplococcus bacterium that is a common colonizer of the human nasopharynx and causes several diseases, such as meningitis, pneumonia, and otitis media. Another common component of the nasopharynx and other host tissues are microplastics. Microplastics are formed when plastic products naturally break down or are deliberately created for use in cosmetics. Microplastics have become ubiquitous in the modern world and there is still little known about their effects on bacterial virulence and invasion. Research has shown that macrophages will uptake large amounts of microplastics and that microplastics in the intestine impact bacterial function. Since S. pneumoniae and microplastics interact in the same niche in host tissues, this may alter S. pneumoniae virulence. We hypothesize that the presence of microplastics affects the immune response to S. pneumoniae infection. To examine these relationships between the immune response and S. pneumoniae we used murine models with and without the presence of microplastics with different strains of S. pneumoniae and microplastic exposure conditions. We also examined immune cell recruitment in murine bronchoalveolar lavage fluid through flow cytometry analysis. Single cell RNAseq analysis was also performed in mice exposed to microplastics compared to a control group. Following infection, we have observed a significantly higher density of bacteria in the lungs of mice that have acute microplastic exposure when strain Tigr4 was used, but the inverse was observed in the strain EF3030. We observed differences in M1 and M2 macrophages based on the presence of microplastics. Preliminary scRNA seq indicates variations in immune cell populations. Our findings suggest that microplastics influence the host immune response to S. pneumoniae infection in a strain-dependent manner. The increased bacterial burden observed in mice infected with the Tigr4 strain under acute microplastic exposure indicates that microplastics may enhance virulence or impair host clearance mechanisms. Conversely, the reduced bacterial density in EF3030 infections suggests a strain-specific interaction with microplastics and host immunity. The shifts in M1 and M2 macrophage populations, along with preliminary scRNA-seq data showing changes in immune cell composition supporting the hypothesis that microplastics modulate innate immune responses. These results highlight the importance of considering microplastics as emerging factors that can influence pathogen behavior and host-pathogen interactions, warranting further investigation into the mechanisms underlying these effects.

CONTRIBUTION OF GROUP B STREPTOCOCCAL ADHESIN BSPC TO NEONATAL INTESTINAL COLONIZATION AND INTERACTIONS WITH *CANDIDA ALBICANS*

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Streptococcus agalactiae, or Group B Streptococcus (GBS) is the leading cause of neonatal meningitis worldwide. Though GBS benignly colonizes the intestinal and female reproductive tract in 30% of healthy adults, exposure to GBS can lead to invasive disease in neonates. Prophylactic intrapartum antibiotics have significantly reduced the incidence of early-onset disease, but late-onset disease, occurring 7 to 90 days post birth, currently has no preventative measures. In these cases, colonization of the neonatal GI tract can lead to serious invasive disease with ongoing neurological sequalae in 50% of survivors. 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, serving to promote 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 Δ*bspC* mutant in the ST-17 strain COH1 displays significantly impaired adherence to both Caco-2 and primary human infant colonic epithelial cells compared to its wildtype counterpart. 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, indicating the importance of this interaction in BspC-mediated adherence. In a neonatal murine meningitis model, mice infected with Δ*bspC* survived significantly better than wildtype infected mice. Δ*bspC*-infected mice also displayed reduced intestinal bacterial burden, supporting a role for BspC in colonization *in vivo*. Interestingly, BspC has been shown to contribute to GBS interactions with the fungus *Candida albicans*, with which it is commonly coisolated from the vaginal tract. *C. albicans* is an opportunistic pathogen that colonizes the neonatal intestinal tract and is also responsible for significant invasive disease in premature neonates. Our experiments have shown that the presence of *C. albicans* enhances GBS adherence to intestinal epithelial cells, and future work aims to determine whether coinfection with *C. albicans* enhances intestinal colonization in vivo. **This work suggests that BspC contributes to GBS colonization in the neonatal intestinal tract through both direct interaction with intestinal epithelial cells and indirectly through interaction with *C. albicans*, leading to the opportunity for progression to invasive disease. Future studies aim to target BspC therapeutically to inhibit GBS intestinal colonization.**

S. AUREUS α-TOXIN RESHAPES THE $CD4^+$ T CELL RESPONSE BY SILENCING LOW-AFFINITY TCR ENGAGEMENT

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Staphylococcus aureus persists as a dire threat to global health, remaining a leading cause of lethal bacteremia and sepsis. Worldwide, over 1 million deaths annually can be attributed directly to *S. aureus* infection. Further complicating public health efforts, previous attempts to formulate a vaccine have failed to elicit robust and durable host immune memory. Therefore, gaining insight into the cellular and molecular mechanisms that this pathogen employs to thwart the host immune response is key for the design of a successful vaccination strategy. From prior murine studies, the pore-forming α-hemolysin (Hla) has been identified as a primary *S. aureus* toxin that impairs the function of antigen-specific $CD4^+$ T cell memory and effector populations. T cell activation is dependent upon the binding of a peptide antigen to the T cell receptor, which triggers an influx of Ca^{2+} ions necessary for an appropriate T cell response. The affinity of this antigen-TCR interaction thus influences downstream T cell priming. We have found that Hla induces rapid T cell membrane depolarization, disrupting the electrochemical gradient crucial for Ca^{2+} influx. However, the resultant effects of varied antigen-TCR affinity in the context of Hla exposure have not been elucidated. In the present study, we hypothesize that Hla-mediated depolarization negates calcium-dependent signaling downstream of low-affinity TCR engagement, skewing the immune response toward high-affinity interactions. This effect is predicted to limit the host immune repertoire. Utilizing an in vitro OTII-OVA model, we were able to selectively manipulate TCR affinity by stimulating isolated $CD4^+$ OTII T cells with antigen-presenting cells (APCs) pulsed with high-affinity $OVA_{323-339}$ peptide or a low-affinity OVA_{H331R} mutant. T cell activation was assessed using surface CD69 expression, a calcium-dependent early activation marker. We found that while Hla attenuated activation in high-affinity antigen conditions, it significantly suppressed CD69 expression in response to OVA_{H331R}. This supports our proposed model that Hla-induced depolarization impairs calcium entry and dampens T cell responses, effectively rendering low-affinity antigens immunologically silent regarding host T cell recruitment. Our work defines a critical barrier to vaccine efficacy against *S. aureus* and highlights a previously unrecognized strategy of immune evasion. With the association of membrane depolarization to the alteration of TCR signal strength and calcium-dependent T cell activation, we reveal a mechanism by which Hla is able to distort the adaptive immune response. This insight has broad implications for *S. aureus* vaccine design, suggesting that early-life immune strategies must address Hla and the signaling function of the T cell compartment to preserve an effective memory response.

A TRANSCRIPTIONAL REGULATOR INDUCED IN MYCOBACTERIAL GRANULOMAS INFLUENCES BACTERIAL PHYSIOLOGY AND DISEASE PROGRESSION

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Tuberculosis is the leading cause of death from a single infectious agent worldwide, but the bacterial and host factors that determine disease trajectory are incompletely understood. Linking the genomic features of specific *Mycobacterium tuberculosis* strains to differences in clinical presentation and outcome can provide insight into key steps in mycobacterial pathogenesis and host response. We identified a novel strain of *M. tuberculosis* from an apparently immunocompetent patient who presented with disseminated TB, including bacterial spread from the lungs to lymph nodes, the parotid gland, and the intestine. Whole genome sequencing identified a private null variant in the conserved transcriptional regulator Rv0260c that may account for at least parts of the clinical phenotype. Through dual RNA-seq from microdissected granulomas in the zebrafish-*Mycobacterium* model we found that its close *Mycobacterium marinum* ortholog is one of the most highly upregulated genes in mature granulomas. In *M. tuberculosis*, Rv0260c has also been reported to be transcriptionally upregulated under stress conditions, including hypoxia and acidic pH. To understand the biological function of this gene in mycobacterial infections and the consequences of loss-of-function mutations, we examined *M. marinum* mutants in the zebrafish model and using a high-throughput phenotypic cell profiling platform. We found *in vivo* alterations in bacterial containment within granulomas in the mutants as well as changes to bacterial physiology, including alterations in cell length and patterns of DNA condensation. Ongoing work aims to further characterize the response of the mutant to specific stress conditions within the granuloma and to shed light on how this conserved transcriptional regulator influences bacterial replication, immune evasion, persistence and dissemination.

OVERCOMING ITACONATE RESTRICTION PERMITS *SALMONELLA* TYPHI INFECTION IN THE MOUSE

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Salmonella enterica serovar Typhi (*S*. Typhi) is a human-specific pathogen that causes typhoid fever, which affects 21 million people each year and results in an estimated 200,000 deaths. As a human-restricted pathogen, there are limited mouse models available to study mechanisms of *S*. Typhi pathogenesis, as unlike human macrophages, mouse macrophages do not support intracellular growth of *S*. Typhi. One difference between mice and humans is the production of the host antimicrobial metabolite itaconate, which is present in activated murine macrophages at 100-fold higher concentrations than in human macrophages (8 mM and 0.06 mM, respectively). *S*. Typhimurium encodes three different mechanisms that enable it to evade itaconate-mediated restriction: two type-III secretion system-2 effectors SopD2 and GtgE, and the ripRCBAlgl operon, which encodes an itaconate degradation pathway. Remarkably, all three of these functions are absent from the genome of *S*. Typhi. Previous work has shown that disruption of itaconate delivery to the *Salmonella*-containing vacuole in mice deficient for the biogenesis of lysosome-related organelle complex 3 (BLOC-3) (*Hps4*-deficient mice) increases permissiveness to intraperitoneal *S*. Typhi infection. Despite this advancement there remains no mouse model for oral *S*. Typhi infection, which is the natural infection route in humans. Here we show that in *S*. Typhimurium, deletion of *ripRCBAlgl* promotes oral *S*. Typhimurium infection of mice via evasion of itaconate restriction. Further, introduction of *ripRCBAlgl* into the *S*. Typhi genome enhanced the survival of *S*. Typhi within bone marrow derived macrophages isolated from mice deficient in BLOC-3. Following oral infection, only *Hps4*-deficient mice, but not congenic controls, that were infected *S*. Typhi containing the itaconate degrading genes (*S*. Typhi::*ripRCBAlgl*), had significant dissemination to systemic organs. These data support that targeting itaconate restriction from both the host and pathogen side promotes *S*. Typhi survival and systemic dissemination in mice following oral infection.

PORPHYROMONAS GINGIVALIS DISTURBS INTERFERON-DEPENDENT ANTIVIRAL RESPONSE, PROMOTING HERPESVIRUS REPLICATION.

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Periodontitis (PD) is a chronic inflammatory disease of the gingiva, with a high prevalence, leading to destruction of the tissue supporting the teeth and eventually to bone loss. *Porphyromonas gingivalis* is one of the pathogens thought to play a key role in the pathogenesis of PD. Clinical reports indicate the significant role of PD in the development of comorbidities, including Herpesviridae infections, but the molecular basis of this phenomenon has not been described. Among possible explanations is the modification of mucosal membranes and subversion of the antiviral response of epithelial cells. Therefore, this study aimed to determine the effect of P. gingivalis infection on the development of viral infections. We uncovered a novel molecular mechanism by which the global interferon-dependent antiviral response is tailored by the cysteine protease of *P. gingivalis* - Kgp. Using gingival keratinocytes and the model of human gingiva we were able to show that lysin-specific gingipain promotes the propagation and penetration into deeper layers of the gingival tissue of HSV-1, which is identified with the highest prevalence in PD patients. These results extend our knowledge of the mechanisms underlying mixed infections and may provide a basis for considering PD as a gateway to viral infection.

Acknowledgments: This project has been supported by National Science Center grant UMO-2021/43/D/NZ6/01906

FLAGELLAR SWITCH INVERTED REPEATS IMPACT HETEROGENEITY IN FLAGELLAR GENE EXPRESSION AND THUS *CLOSTRIDIOIDES DIFFICIL*E RT027/MLST1 VIRULENCE

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Clostridioides difficile is the leading cause of hospital-acquired infection in the United States. *C. difficile* RT027/MLST1 strains have been associated with increased toxin production and more severe disease. However, the ability of these strains to cause disease varies from asymptomatic to severe diarrhea to lethal in patients, which complicates clinical disease management. To gain insight into the mechanisms underlying this differential virulence, we conducted comparative genomic, transcriptomic, and phenotypic comparisons of a panel of RT027 clinical isolates. These analyses suggested that isolates with greater heterogeneity in flagellar gene expression exhibit greater virulence *in vivo*. *C. difficile* flagellar genes are phase-variably expressed due to the site-specific inversion of the *flgB* 5'UTR region, which reversibly generates ON vs. OFF orientations for the flagellar switch. We found that isolates with longer inverted repeats (IR) sequences in this upstream region (6A/6T) exhibit greater heterogeneity in flagellar gene expression (60-75% ON) and greater virulence than isolates with shorter IRs (>99% ON or OFF). Moreover, exchanging a 6A/6T IR sequence with a shorter 5A/5T sequence reduced the virulence of a typically virulent strain. Taken together, our results reveal that shorter IR types decrease flagellar switching, which leads to more uniform flagellar gene expression within a population and decreased virulence *in vivo*. Our findings may help explain the variable disease outcomes observed in *C. difficile* patients and provide direct evidence that generating phenotypic heterogeneity can alter disease outcomes.

ENGINEERING MULTI-SPECIFIC ANTIBODIES TO IMPROVE BROAD NEUTRALIZATION OF *CLOSTRIDIOIDES DIFFICILE* TCDB

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Clostridioides difficile infection (CDI) is an urgent antibiotic-associated health threat responsible for high morbidity and mortality with limited treatments and a 30% recurrence rate. CDI symptoms are largely mediated by a *C. difficile* exotoxin, TcdB, making it an attractive therapeutic target. However, TcdB sequences vary across strains, forming distinct subtypes. TcdB1, TcdB2, and TcdB3 are the most clinically relevant, as they cause the most severe disease in animal models and are the most prevalent in strains infecting humans. The sequence variation between subtypes presents a challenge for pan-neutralizing monoclonal antibody (mAb) therapeutics. Further, there is a critical need for new mAb therapeutics to treat CDI, since the only FDA-approved one, ZINPLAVA™ (bezlotoxumab), was discontinued in January 2025. Nanobodies are an alternative to conventional mAbs, with multiple advantages including their small size and high target specificity. We have a panel of TcdB-neutralizing nanobodies targeting different epitopes which were fused to human antibody Fc backbones. A “knobs-into-holes” technique enabled us to engineer multi-specific nanobody-Fc fusion proteins which we hypothesized would enhance TcdB neutralization. We currently have three bi-specifics and one tri-specific. All showed greater neutralization than bezlotoxumab *in vitro* against TcdB1, 2, and 3 with IC_{50} values in the picomolar range. In addition, we have tested each bi-specific as a prophylactic in a murine model of CDI against representative strains for TcdB1, 2, or 3, and the tri-specific against the same TcdB1-containing strain. In an infection with a TcdB1 representative strain, one bi-specific and the tri-specific showed protection from initial infection weight loss, and the tri-specific improved weight recovery. Survival against this strain was significantly improved by two bi-specifics and the tri-specific. Against a TcdB2-containing strain, those same two bi-specifics showed protection, with one continuing to protect at a lower dose. When infected with a TcdB3 representative strain, both bi-specifics also reduced histopathology severity. The data showcase the potential of multi-specific nanobody-Fc’s capable of neutralizing across TcdB subtypes as alternative therapeutics for CDI.

CANONICAL GENETICS APPROACH SPARKS MUTATION AT HOTSPOTS IN THE *LEGIONELLA* GENOME

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A common strategy for studying microbial virulence factors is reverse genetics, a method where a gene or genes of interest are deleted from the genome and phenotypic effects are analyzed. Clone selection involves their exposure to a series of selective pressures, including antibiotics, and growth on non-natural surfaces. Using *Legionella pneumophila* as a model, we found that bacteria respond to these stressors during allelic exchange by acquiring unsought background mutations at an elevated rate. We found that nearly 75 percent of all tested clones had acquired additional nonsynonymous mutations, with more than 25 percent of mutations found in the genes encoding the LetA/LetS two-component system. These mutations were overwhelmingly predicted to cause loss-of-function peptide truncations. Tracking a frequently observed frameshift mutation in *letA* throughout the allelic exchange protocol at single colony resolution revealed that this mutation arose steadily at each passage step and at a reproducible frequency. Clones with this *letA* mutation phenocopied strains with a *letA* in-frame deletion, including attenuated intracellular replication, thus having the potential to skew conclusions of downstream experiments if not surveilled. Notably, mutation frequency during allelic exchange was dramatically reduced in strains with a non-functional Dot/Icm type IV secretion system (T4SS), suggesting that unregulated leakage of metabolites through the T4SS is problematic. These data demonstrate that the genome of virulent *Legionella* is prone to mutations in response to stress such as genetic manipulation in the laboratory setting.

ENTEROCOCCUS FAECALIS INTESTINAL BLOOMS ARE ASSOCIATED WITH DEATH IN A GNOTOBIOTIC MOUSE MODEL OF ANTIBIOTIC-INDUCED NEONATAL SEPSIS

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Background: *Enterococcus faecalis* colonizes the infant gut and is a leading cause of Gram-positive bloodstream infection (BSI) and sepsis in preterm infants. Recent work has shown that the exact BSI-causing strain of *E. faecalis* can be identified in the gut before sepsis in preterm infants, suggesting gut-to-bloodstream translocation as a pathomechanism for BSI. This outcome is likely mediated by interplay between the immune system, microbiome composition, and strain-specific *E. faecalis* pathogenic potential. In the NICU, extended antibiotic exposure increases sepsis risk. We hypothesized that certain antibiotics allow *Enterococcus* overgrowth and infection by *Enterococcus* that produces GelE, a gelatinase that increases dissemination from primary infection sites.
Methods: We colonized Germ-free dams with preterm infant stools without *E. faecalis* (Microbiome D), gelatinase negative *E. feacalis* (Microbiome A), or gelatinase positive *E. faecalis* (Microbiomes B and C). Their pups were treated with antibiotics from day of life 10-13. We determined microbiome compositions with metagenomic sequencing, then isolated and sequenced *E. faecalis* from preterm stools. We assembled genomes with SPAdes and annotated them with Bakta. Gelatinase activity was measured by growing isolates on BHI agar with 3% gelatin.
Results: During meropenem treatment, 25% of microbiota B- and 32% of C-colonized pups died compared with 4% of microbiota A- or D-colonized pups ($p<0.05$ for A/D vs B/C). Microbiota B- and C-colonized pups had dysregulated serum cytokines compared to microbiota A-humanized pups. Mice colonized with a mix of Microbiomes A and B phenocopy Microbiome B-colonized pups (36% death). Treatment with vancomycin protected against death in Microbiome A/B mixtures ($p<0.05$). Meropenem led to high intestinal loads of *E. faecalis* compared to vancomycin (8.2x104 CFU/mL vs 6.7x101 CFU/mL, $p<0.005$). Microbiome A *E. faecalis* does not have measurable gelatinase activity, while *E. faecalis* from Microbiomes B and C do.
Conclusions: When neonatal mice are colonized with microbiotas that include GelE-producing *E. faecalis*, meropenem treatment leads to increases in *E. faecalis* intestinal abundance, systemic immune dysregulation, and death. While the exact pathomechanism is unknown, correlation between gelatinase activity and death merits further work to understand the host-microbe interaction that leads to sepsis.

SYSTEMATIC IDENTIFICATION OF BACTERIAL FACTORS DRIVING *STAPHYLOCOCCUS AUREUS* INTRACELLULAR BEHAVIOR IN NON-PROFESSIONAL PHAGOCYTES

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Staphylococcus aureus is a major human pathogen responsible for a broad spectrum of life-threatening nosocomial and community-acquired infections. Although traditionally described as an extracellular pathogen, accumulating evidence from in vitro and in vivo studies establishes *S. aureus* as a facultative intracellular pathogen. Supporting this, our recent work revealed that the intracellular lifestyle is prevalent among *S. aureus* clinical isolates from patients with bone/joint infections, bacteraemia, and infective endocarditis. *S. aureus* intracellularity contributes to immune evasion, bacterial dissemination, and failure of antibiotic therapies. Identifying and characterising the bacterial factors enabling *S. aureus* to thrive intracellularly is therefore critical. Here, we screened a comprehensive collection of 1,920 *S. aureus* mutants (Nebraska transposon mutant library), encompassing virtually all the non-essential bacterial genes, to assess the impact of each bacterial factor on invasion, replication, persistence, and host cytotoxicity in epithelial cells, across five timepoints of infection (0.5 to 48 hours post-infection). We identified 73 bacterial factors that strongly modulate these processes, including mutants eliciting multiple phenotypes. Notably, several of these factors have not been previously characterized, and the large majority has not been implicated in *S. aureus* intracellularity. Among these, we characterized the nicotinamidase PncA as a novel regulator of the agr system through the modulation of the bacterial redox state, with profound effects on *S. aureus* virulence. This work provides a comprehensive and systematic analysis of *S. aureus* factors critical for its intracellular behavior, with important implications for the development and optimization of antimicrobial therapies targeting this resilient bacterial population.

A QUORUM SENSING SYSTEM OF GROUP A STREPTOCOCCUS THAT INHIBITS INNATE INFLAMMATORY SIGNALING

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The Rgg2/Rgg3 quorum sensing (QS) system of Group A Streptococcus (GAS) controls expression of 12 genes in response to short hydrophobic peptide pheromones (SHPs). This system is among the most highly up-regulated genetic program following the inoculation of GAS in an intact-skin model of infection. Because the system is regulated by both positive (Rgg2) and negative (Rgg3) means, we have generated mutants that lock the system in either 'QS-ON' (*Δrgg3*) or 'QS-OFF' (*Δrgg2*) states that mimic gene expression levels seen when GAS is provided a synthetic SHP (producing QS-ON) or a reversed-sequence peptide (rev-SHP, bacteria remain in QS-OFF state). Skin infections by WT or QS-ON strains develop purulent blisters and substantial inflammation, whereas the QS-OFF mutant is attenuated and the skin heals after initial signs of erythema. We find that infection of cultured macrophages with QS-ON GAS, but not QS-OFF, inhibit inflammatory responses induced by Pathogen-Associated Molecular Patterns and Toll-like receptor agonists. It is our objective to identify the mechanisms preventing macrophage activation, asking what aspects of gene expression are disrupted in the macrophage and how do the bacteria induce this effect. The conventional NFκB signal transduction pathway connecting TLRs to transcription factor nuclear translocation is functional in both QS-ON and QS-OFF infection conditions, indicating that the block in gene expression occurs in the nucleus. Phosphoproteomic analysis supports this conclusion and suggests epigenetic regulators and a possible differential activity of the c-Rel subunit may account for suppressed gene expression. The QS-induced 10-gene qim operon is required for immune suppression and its expression is associated with the presence of a ribitol-N-acetylglucosamine modification on the cell wall. We hypothesize this modification elicits the suppressive effects on macrophages, possibly through immunosuppressive receptors or regulatory networks that have been identified by a CRISPR mutagenesis screen.

THE ROLE OF MGTC IN REGULATING HYPERMUCOVISCOSITY IN *KLEBSIELLA PNEUMONIAE*

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Klebsiella pneumoniae infections pose a growing threat to human health due to increasing multidrug resistance and virulence, prompting the CDC to classify it as a pathogen in urgent need of new therapeutic strategies. With the recent emergence of virulent strains in this historically opportunistic species, it is essential to better understand the genetic factors that contribute to virulence. We discovered that a host defense peptide produced by bovine neutrophils significantly induces the expression of *mgtC*, a virulence-associated gene known in *Salmonella enterica* to promote intracellular survival. To date, the function of MgtC in *K. pneumoniae* has not been characterized. To investigate the function of MgtC in the hypervirulent *K. pneumoniae* strain NTUH-K2044, we generated an isogenic mutant lacking *mgtC* and introduced an inducible vector system to control *mgtC* expression using IPTG. Induction of *mgtC* in NTUH-K2044 led to a significant reduction in both hypermucoviscosity and biofilm formation. When assessed on Congo red agar, mgtC induction resulted in reduced polysaccharide production and metabolic activity, consistent with prior findings in *S. enterica* showing that MgtC inhibits FoF1 ATP synthase activity. In *S. enterica*, MgtC has also been linked to decreased cellulose production through reduced levels of the allosteric activator c-di-GMP. However, its impact on sedimentation resistance and mucoidy has not been studied, likely due to the lack of research in highly mucoid bacteria. Interestingly, while *mgtC* induction in *K. pneumoniae* reduced cellulose production and resulted in translucent colonies, it did not alter overall capsule levels, as measured by glucuronic acid quantification and SDS-PAGE visualization. These findings suggest that the observed decrease in hypermucoviscosity may be attributed to reduced cellulose and fimbriae rather than capsule production in NTUH-K2044. Our results demonstrate that MgtC is functional in *K. pneumoniae* and may play a role in modulating bacterial metabolism under environmental stress.

RIBOSOMAL PROTEIN PARALOGS AND ZINC HOMEOSTASIS IN *NEISSERIA GONORRHOEAE*

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Treatment of *Neisseria gonorrhoeae* (Gc) is complicated by antibiotic resistance and the lack of effective vaccine. One potential therapeutic target is Gc zinc acquisition. Zinc availability varies in the human host, and it is unknown how Gc maintains internal zinc homeostasis. In other bacteria, non-zinc-binding ribosomal proteins (r-proteins) replace zinc binding paralogs on the ribosome under zinc limitation; this process is hypothesized to enable bacterial survival by liberating zinc or altering ribosome function. Gc encodes two sets of paralogous r-proteins, *rpmE*/*E2* and *rpmJ*/*J2*. RpmE and RpmJ contain a CXXC predicted zinc-binding motif (C+); RpmE2 and RpmJ2 are predicted to be zinc-independent (C-). This project aims to define the role of RpmE/E2 and RpmJ/J2 in zinc-limited Gc. We hypothesized that in zinc-limited Gc C- r-proteins are induced and replace their C+ paralogs on the ribosome, enabling Gc growth under zinc limitation by liberating free zinc or altering ribosome function. To test this, we investigated the following in zinc-replete and zinc-limited Gc: transcriptional regulation of *rpmE*/*E2* and *rpmJ*/*J2*, RpmE and RpmE2 protein production, C+ and C- r-protein presence in isolated ribosomes and polysomes (mRNA-bound ribosomes), and growth of RpmE/RpmE2 locked strains and strains lacking RpmE or RpmE2/RpmJ2. We found that the *rpmE2-rpmJ2* co-transcript is induced under zinc limitation by Zur derepression and RpmE2 is produced. Expression of *rpmE* and *rpmJ* is zinc-independent, and RpmE production is zinc-independent. RpmE is not degraded with zinc limitation; the Gc model for r-protein alternation differs from bacteria in which RpmE is degraded to liberate zinc. Additionally, RpmE and RpmE2 are detected in zinc-limited Gc ribosomes. Gc lacking RpmE exhibit a zinc-independent growth defect rescued by complementation with RpmE but not RpmE2, and Gc lacking RpmE2 and RpmJ2 are less susceptible to zinc limitation than WT Gc. Cryo-EM 3D reconstructions of RpmE- and RpmE2-containing ribosomes show that both types of ribosomes are bound to tRNA and mRNA, indicating ongoing translation. Our work demonstrates that C- r-proteins are induced in zinc-limited Gc and may drive reduced growth. However, RpmE2-ribosomes exhibit similar translation competency to RpmE-ribosomes. We hypothesize that r-protein alternation drives changes in ribosome function that suppress Gc growth and translation under zinc limitation to enable persistence. We are currently exploring how Gc r-protein alternation affects translation and Gc persistence during zinc-limited infection. Investigating the importance of r-proteins for Gc persistence in the host could reveal new targets to improve treatments for drug-resistant gonorrhea.

A SUBSET OF GROUP B STREPTOCOCCAL SECRETED EFFECTORS IMPAIRS VAGINAL COLONIZATION

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The opportunistic pathogen, group B Streptococcus (GBS), asymptomatically colonizes the female genital tract of approximately 25% of pregnant people. GBS ascension to the uterus and/or placenta has been shown to increase the risk of adverse pregnancy outcomes. GBS remains a leading cause of neonatal bacteremia and meningitis due to maternal transmission in utero or during birth; therefore, understanding the mechanisms by which GBS persists in the female genital tract is integral to preventing subsequent disease. Type VII secretion systems (T7SS) encoded by Actinobacteria and gram-positive bacteria have been shown to promote virulence, modulation of immune responses, and interbacterial competition. We found previously that four distinct subtypes of GBS T7SS exist across cohorts of clinical isolates, each encoding a unique repertoire of secreted effectors. Using a GBS isolate encoding the common subtype I T7SS, we found that loss of the T7SS resulted in decreased invasion of the murine female genital tissues compared to a parental strain, indicating subtype I effectors play an essential role in colonization. Interestingly, using a GBS strain encoding a subtype III T7SS, the loss of T7SS increased the persistence of GBS in the vaginal lumen as well as increased invasion of the murine female genital tissues compared to a parental stain, indicating that subtype III effectors may impair colonization. However, the mechanism by which this subset of secreted effectors promotes GBS mucosal clearance remains unknown. In this project, we examine the impact of GBS T7SS subtype III effectors on the vaginal epithelium, mucosal immune response, and vaginal microbiota using in vitro and in vivo models of vaginal colonization. We hypothesize that effectors secreted by the subtype III GBS T7SS may elicit inflammatory responses in the vaginal tract, resulting in parental GBS clearance. Understanding the mechanism by which GBS is cleared from the vaginal tract may inform future therapeutic strategies to limit GBS persistence and neonatal transmission.

LEVERAGING ECOLOGY FOR *CLOSTRIDIOIDES DIFFICILE* MRNA VACCINE DESIGN

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Clostridioides difficile is a spore-forming gastrointestinal (GI) pathogen that causes significant morbidity and mortality worldwide. Despite the global disease burden of *C. difficile* infection (CDI), current therapies are inadequate and lead to high rates of recurrence. *C. difficile* lives a complex lifestyle and causes infections in a wide array of diverse patient populations, including healthy patients and patients with comorbidities such as inflammatory bowel disease and cancer. In addition, *C. difficile* asymptomatically colonizes infants at high rates. As the gut microbiota, metabolites, and host responses differ across age and disease state, effective design of novel therapeutics requires a better understanding of how *C. difficile* colonizes different niches across various patient populations. To this end, we are defining the ecological niche of *C. difficile* across the spectrum of disease severity and age using novel CDI mouse models, transcriptomics, and advanced imaging metabolomics. By leveraging this multi-omic strategy, we are systematically identifying the genetic determinants of *C. difficile* colonization and competition with the host microbiota in distinct GI niches. Notably, we have identified several genes that are highly expressed across contexts, including many spore coat proteins and nutrient importers required for vegetative cell growth. Leveraging this ecology-forward strategy, we further report the generation of the first multivalent mRNA-LNP vaccines to target these systems and provide updates on our work towards developing a *C. difficile* vaccine that will be effective in preventing CDI in all patient populations.

THE INTERPLAY BETWEEN TYPE I AND II INTERFERONS IN REGULATING COSTIMULATORY MOLECULE EXPRESSION ON ALVEOLAR MACROPHAGES DURING PULMONARY INFLAMMATION

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The immune response to external stimuli in the lungs must balance inflammation to maintain gas exchange function. Alveolar macrophages (AMs) are the first immune cell in the lungs to sense external stimuli and establish appropriate inflammatory responses while controlling invading pathogens such as *Mycobacterium tuberculosis* (Mtb). However, we currently lack an understanding of specific mechanisms regulating inflammation in AMs. To fill this gap in knowledge, we are using a new AM model known as fetal liver-derived alveolar-like macrophages (FLAMs), which recapitulate AM biology and allow for genetic studies that remain challenging in primary AMs. While AMs are antigen presenting cells, we found both AMs and FLAMs express low surface expression of the costimulatory molecules CD80 and CD86, even after cellular activation with the cytokine interferon gamma (IFNγ). This results in poor T cell activation by these cells and we found that the restrained expression of CD80/CD86 is controlled by the kinases glycogen synthase kinase 3α and 3β (GSK3α/β). Surprisingly, we also found that chemical inhibition of GSK3α/β in FLAMs activated with IFNγ, induced a significant type I IFN response that was reversed by the addition of a PPARγ agonist or a mitochondrial targeted antioxidant. These data suggest a model that connects GSK3α/β, lipid metabolism, mitochondrial stability, and inflammation in AMs. To understand the underlying regulation of these pathways we conducted co-culture experiments that revealed that IFNβ1 drives robust CD86 expression following IFNγ-activation and GSK3α/β inhibition. However, CD80 was not regulated similarly. While recombinant IFNβ1 stimulation during GSK3α/β blockade upregulated both CD86 and CD80 we found that IFNγ uniquely reduced CD80 expression but not CD86. Given the importance of regulating inflammatory responses in the lungs, and the central role of CD80/CD86 in tuning T cell responses, our work highlights new regulatory mechanisms of AM-mediated inflammation and T cell activation. In ongoing studies, we are defining the pathways that regulate GSK3α/β function, IFNβ1 and IFNγ responses, in addition to CD80 and CD86 expression in AMs. We are testing how these interconnected pathways modulate the host response following Mtb infection and the impacts on T cell activation and pulmonary disease. Taken together, these studies are defining how AMs control the lung inflammatory milieu to restrict pathogens while preventing deleterious lung damage.

PORPHYROMONAS GINGIVALIS-MEDIATED REACTIVATION OF LATENT HERPES SIMPLEX VIRUS

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Herpes simplex virus (HSV) affects over 60% of the global population, typically persisting asymptomatic in its latent form within neuronal cells. Reactivation of latent viruses is as significant as their propagation, as recurrent cycles of HSV latency and reactivation may contribute to the development of neurodegenerative diseases. Recent evidence has demonstrated that *Porphyromonas gingivalis*—a key pathogen implicated in the pathogenesis of periodontitis—and its virulence factors are present in the brain tissue of patients with Alzheimer's disease. Moreover, previous studies have shown that *P. gingivalis* can reactivate latent forms of Epstein-Barr virus (EBV) and human immunodeficiency virus (HIV). Given our earlier findings that *P. gingivalis* virulence factors, gingipains, influence HSV-1 propagation, we aimed to determine whether this bacterium could also trigger reactivation of alphaherpesviruses. Our data reveal that the gingipains, induces reactivation of latent HSV in a human neuronal cells. These findings establish a molecular link between periodontal disease and neurodegeneration, providing critical insights into the potential mechanisms underlying their comorbidity.
Acknowledgments: This project has been supported by National Science Center grant UMO-2021/43/D/NZ6/01906.

WITHIN-HOST GENETIC DIVERSITY OF *SALMONELLA ENTERICA* DURING ACUTE HUMAN INFECTION

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Salmonella enterica is a globally significant pathogen and a leading cause of foodborne illness, with both typhoidal and non-typhoidal serovars responsible for millions of infections and hundreds of thousands of deaths annually. Despite this public health burden, our understanding of within-host genetic diversity during acute infection remains limited. Such knowledge is critical for elucidating bacterial evolution, transmission dynamics, and antimicrobial resistance.

To investigate how genetically diverse *S. enterica* populations are within individual patients, we performed whole-genome sequencing on 10 blood or stool isolates each from 23 patients with acute salmonellosis. All isolates from the same patient belonged to the same serovar and were separated by no more than 10 single nucleotide polymorphisms (SNPs), indicating a common infection source. However, stool-derived isolates exhibited significantly more genetic variation—including SNPs, large structural variants, and differences in plasmid content—than blood-derived isolates, suggesting a bottleneck effect during systemic dissemination.

Notably, we observed that same-patient isolates often varied in their predicted antibiotic resistance profiles, particularly for aminoglycosides. Phenotypic testing of the isolates from these patients found that this differential presence of aminoglycoside resistance conferring genes translated to real differences in streptomycin susceptibility. These findings challenge the common practice of relying on single-colony isolates for diagnostics and surveillance, highlighting that clinically relevant diversity can exist within a single host even during acute infection.

Our results reveal that *S. enterica* populations can be genetically heterogeneous during acute infection, with meaningful implications for antimicrobial resistance prediction, treatment strategies, and understanding the evolutionary trajectory of bacterial pathogens. Recognizing and accounting for within-host diversity will be increasingly important in the era of genomic medicine and rising antimicrobial resistance.

HOST INFLAMMATION PLAYS A CRITICAL ROLE IN SPEB TRANSCRIPTIONAL REGULATION IN GROUP A *STREPTOCOCCUS*

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Group A *Streptococcus* (GAS) infections are a major burden to the healthcare system in the United States and globally. Infections are often mild, such as pharyngitis, but can rapidly progress into invasive infections, including toxic shock syndrome and necrotizing fasciitis. Invasive infections are often difficult to treat, due to inflammation and tissue destruction, which impairs antibiotic penetration to the infection site. GAS drives inflammation through the coordinated expression of virulence factors, including the cysteine protease SpeB. SpeB activity is essential for establishing skin infections by cleaving bacterial and host proteins, resulting in dissemination and disease progression. Growth phase, pH, and nutrients are important in regulating SpeB. The host defense peptide LL-37 also regulates SpeB and determines whether expression is on or off, through the two-component system CovRS. Neutrophils undergo inflammatory cell death and are a main source of LL-37 release during GAS infections. While in vitro expression is well characterized, dynamics of SpeB regulation remain unclear within these inflammatory environments. Complementary genetics and fluorometry techniques were used to examine SpeB regulation during infection with neutrophils collected from healthy donors. SpeB expression was characterized during infections with wild-type and knockout strains of CovRS using flow cytometry. Furthermore, SpeB regulatory patterns were characterized in vivo using a neutrophil depleted mouse intradermal infection model. Results suggest that in the presence of neutrophils, CovRS regulation is mediated by LL-37. Moreover, SpeB expression is mediated by the presence or absence of inflammatory markers. Flow cytometry data confirms these results in vivo. Neutrophil depletion during intradermal infections alters SpeB regulatory patterns within the population. Results indicate that host inflammation plays a key role in SpeB activity during GAS infections. Further, SpeB regulation is much more complex than previously suggested, pinpointing key drivers associated with GAS induced inflammation that leads to morbidity associated with invasive infections and antibiotic failure.

THE IMPACT OF MELATONIN IN THE REGULATION OF EHEC VIRULENCE

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Enterohemorrhagic *Escherichia coli* (EHEC) is a foodborne human pathogen colonizing the colon, causing global outbreaks of bloody diarrhea and potentially leading to life-threatening hemolytic uremic syndrome (HUS). Despite the pathogen's low infectious dose and severe complications, the molecular mechanisms underlying EHEC pathogenicity are not completely understood. EHEC employs a type III secretion system (T3SS), encoded by the locus of enterocyte effacement (LEE) pathogenicity island, to form attaching and effacing (A/E) lesions. The virulence of EHEC is modulated by the environmental signals within the gastrointestinal (GI) track. Among these signals, we have already shown that serotonin and indole decrease EHEC virulence. Since serotonin and indole are tryptophan derivative neurotransmitters, here we explored the effect of melatonin, another tryptophan-derived molecule predominant in the GI. Our data from post-transcriptional and transcriptional studies show upregulation in LEE expression by melatonin treatment. The transcriptomic studies further confirm that melatonin leads to up-regulation of LEE gene expression, expression of T3SS effector and structure proteins, while chemotaxis and flagellin genes are down-regulated in response to this neurotransmitter. This suggests that EHEC senses melatonin as an environmental cue and responds by upregulating virulence gene expression, enhancing epithelial adherence, and A/E lesions through a switch to a non-motile state. Interestingly, we also observed a significant downregulation of *tnaA*, responsible for converting tryptophan to indole. Inasmuch as indole is known to suppress EHEC virulence, these findings suggest that melatonin enhances virulence by both activating LEE expression and suppressing the indole signaling pathway that decreases virulence expression. To characterize the effect of melatonin in enteric pathogenesis during mammalian infection, *Citrobacter rodentium* is used as a surrogate murine model of EHEC infection. The *in vivo* studies show that mice receiving melatonin in drinking water exhibited higher colonization and reduced survival, suggesting that melatonin worsens the disease caused by *C. rodentium* infection of mice. Upon completion of this work, we will further understand melatonin's novel impact in regulating EHEC virulence and host interactions. This work will provide new insights into host-microbe communication and potential therapeutic strategies by characterizing the crosstalk between EHEC and melatonin.

PROPIONATE LINKED TO LA-1 TREATMENT SIMULTANEOUSLY SUPPRESSES OSTEOARTHRITIS PAIN AND JOINT DAMAGE BY RESTORING MITOCHONDRIAL FUNCTION AND STABILIZING THE AUTOPHAGY PROCESS

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In previous study, we found that inactivated Lactobacillus (LA-1) administration ameliorates osteoarthritis (OA) and increases propionate-producing bacteria. However, the role of propionate in the development and progression on the OA remains unclear and externally administered propionate has limitations in maintaining a stable concentration. In this study, we evaluated the therapeutic potential of propionate in the OA treatment. To investigate the effect of propionate on OA progression, we administered propionate into MIA-induced OA animals. The pain threshold, cartilage damage, and inflammation of the joint synovial membrane were improved by Sodium Propionate. Also, Propionate treatment suppressed mTOR activity, leading to increased nuclear translocation of transcription factor EB (TFEB), a key regulator of lysosomal biogenesis and autophagy. This activation enhanced autophagic flux, restored cellular homeostasis, and reduced necroptosis in chondrocytes. In an OA animal model, Propionate significantly mitigating cartilage damage, inflammation, and pain. These findings highlight the potential of Propionate as a novel therapeutic approach for OA by regulating mTOR-TFEB-mediated autophagy.

Keywords: Propionate, Osteoarthritis, Transcription factor EB, Autophagy

PATTERNS OF HYPERVIRULENT *KLEBSIELLA PNEUMONIAE* GASTROINTESTINAL COLONIZATION AND SYSTEMIC DISSEMINATION

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Hypervirulent *Klebsiella pneumoniae* (hvKP) is an emerging pathogen capable of causing community-acquired infections in otherwise healthy individuals. Growing evidence suggests that gastrointestinal (GI) colonization is a key reservoir that often precedes dissemination and invasive disease. The dynamics of hvKP expansion within the GI tract, dissemination to extraintestinal sites and host clearance mechanisms are not well understood. Using our animal model of hvKP gut colonization, we investigated the population dynamics of hvKP during GI colonization and translocation, by employing a barcoded hvKP library (~50,000 unique 25-nucleotide barcodes inserted at the neutral Tn7 site) derived from clinical isolate hvKP1, paired with the STAMPR (Sequence Tag-based Analysis of Microbial Populations) pipeline. We orally inoculated wild-type C57BL/6 mice with 10^6 CFU of the hvKP1-STAMPR library and collected GI and systemic samples at 24 and 72 hours post-inoculation. High bacterial burden was observed at both time points in the GI and systemic organs, with up to 100-fold higher loads at 72 hours. Despite this, founding populations (unique established clones) in the extraintestinal tissues were ≤ 7 clones, representing less than 0.014% of the input library and suggestive of a tight host bottleneck at the translocation step. In contrast, GI sites showed far greater clonal diversity (~10^4 unique clones; ~20% of the input library). Notably, extraintestinal populations were highly similar across organs (low genetic distance), suggesting that once hvKP translocates through the intestinal barrier, it readily replicates and spreads systemically. Increasing the inoculum dose had minimal impact on clonal diversity in both the GI and extraintestinal sites. To explore the role of the native microbiota in shaping colonization dynamics, we pretreated mice with either a low or a high dose of ampicillin, 24 hours before hvKP inoculation, which led to dose-dependent microbial shifts that influenced both colonization efficiency and clonal selection. Collectively, our findings demonstrate that founding populations establish early, are only modestly influenced by inoculum size, and are shaped by microbiome disruption. These results highlight the bottlenecks controlling hvKP colonization and systemic spread, and ultimately shaping infection outcomes.

INNATE SENSING OF SALMONELLA INTRACELLULAR REPLICATION TRIGGERS CASPASE-8-DEPENDENT MACROPHAGE APOPTOSIS VIA TLR4-TNF SIGNALING PATHWAY

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Salmonella enterica is a gram-negative bacterial pathogen comprising over 2500 serovars that are responsible for over 90 million infections and 100,000 deaths annually worldwide. Despite the diversity of serovars, our understanding of innate immune detection of *Salmonella* infection is based on extensive use of a limited number of strains, primarily of the serovar Typhimurium. *S.*Typhimurium infection of macrophages is believed to induce pyroptosis in murine macrophages primarily via caspases-1 and -11. However, this conclusion is based largely on studies with strains of *S.* Typhimurium that replicate poorly within macrophages. Here we demonstrate using a combination of clinical isolates *Salmonella* enterica that replicate to high levels within murine macrophages that in addition to the established caspase-1/11-dependent pyroptotic response, intracellular replicating Salmonella triggers a previously undescribed pathway of caspase-8 dependent apoptosis. This pathway requires bacterial replication as well as both TLR4 signaling and TNF secretion, which results in subsequent activation of caspase-8 and downstream activation of the apoptotic pore Pannexin-1. Our findings uncover a previously unappreciated mechanism of macrophage innate immune sensing that detects intracellular bacterial replication.

DIETARY FIBER MODULATES SUSCEPTIBILITY TO *CLOSTRIDIOIDES DIFFICILE* INFECTION POST-ANTIBIOTIC TREATMENT

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Clostridioides difficile is an urgent public health threat and the number one cause of antibiotic-associated diarrhea in the United States. While *C. difficile* is commonly considered a nosocomial pathogen, the epidemiology of *C. difficile* infection (CDI) is rapidly evolving. Community-acquired cases of CDI are increasing compared to hospital-associated cases, and understanding the factors that contribute to the risk of CDI is urgently needed. The main risk factor contributing to CDI is recent antibiotic use; however, some individuals remain susceptible to CDI for months post-antibiotic treatment. Importantly, we do not understand why some patients remain susceptible to CDI for such extended time periods. Based on a report of CDI associated with a clinical dietary intervention trial at our hospital, we hypothesized that dietary fiber may modulate susceptibility to *C. difficile* post-antibiotic treatment in patients. To determine if fiber impacts factors associated with colonization resistance to *C. difficile*, we investigated the metabolome and microbiota in human subjects from our trial that were on a low-fiber diet. Moreover, to directly test how fiber impacts CDI susceptibility, we treated mice with fiber-rich or fiber-free diets and quantified CDI susceptibility after antibiotic use. We report that a low-fiber diet leads to increased fecal primary conjugated bile acids in humans, including bile acids known to promote *C. difficile* colonization such as taurocholic acid (TCA). Using our novel mouse model of CDI, we show that a fiber-free diet leads to prolonged and increased susceptibility to CDI that is associated with alterations in bile acids. We further report long-lasting perturbation to the microbiota, highlighted by depletion of *Clostridia* and an enrichment of facultative anaerobes. In ongoing studies, we are using fiber supplementation to promote de-colonization of *C. difficile* in high-risk patients. This work suggests that in the context of antibiotic treatment, diet is a critical, modifiable risk factor for CDI susceptibility.

KLEBSIELLA PNEUMONIAE FACTORS ENHANCING BACTEREMIA HAVE DISTINCT CONTRIBUTIONS TO MACROPHAGE-MEDIATED, OXIDATIVE, AND NITROSATIVE STRESS RESISTANCE

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Klebsiella pneumoniae is a Gram-negative species that is a leading cause of hospital-associated infections. Such infections often result in bacteremia, when bacteria disseminate from initial sites of disease to the bloodstream and colonize filtering organs like the spleen and liver. Previous studies have demonstrated that macrophages play a substantial role during K. pneumoniae infection. While interactions between alveolar macrophages and K. pneumoniae have been described in the context of lung infection, much less is known about interactions between K. pneumoniae and monocyte-derived macrophages, a subset abundant in the lung during pneumonia and present across tissues in systemic infection. Genes utilized by K. pneumoniae to resist macrophage-mediated killing and common forms of stress imposed by innate immune cells remain poorly understood in the context of bacteremia. Here, we investigated the role of 53 previously identified K. pneumoniae bacteremia fitness factors for their role in stress resistance against macrophage-mediated, oxidative, and nitrosative stress. K. pneumoniae bacteremia fitness factors displayed unique contributions to stress resistance. Increased K. pneumoniae hypermucoviscosity generally correlated with lower uptake by macrophages, but the presence of polysaccharide capsule did not enhance intracellular fitness. About 36% of bacteremia fitness factors were required to resist intracellular stress or nitrosative stress, and ~25% were important for resisting oxidative stress. Some K. pneumoniae factors were required to resist oxidative or nitrosative stress but dispensable to resist macrophage-mediated stress. Other factors enhanced intracellular fitness but were specific to either oxidative or nitrosative stress resistance. For example, the mannitol-1-phosphate dehydrogenase enzyme, MtlD, was necessary to resist intracellular and nitrosative stress but dispensable under oxidative conditions. Some factors like PdxA, a member of the vitamin B6 biosynthesis pathway, was required in all three stress environments. Alternatively, some factors were required for intracellular survival but independent from resistance related to oxidative or nitrosative stress. These findings provide new insights into the interactions between K. pneumoniae and common forms of stress elicited by innate immunity, furthering our understanding of the strategies employed by K. pneumoniae to withstand immunological stress during bacteremia.

COMBINATORIAL EFFECTS OF TRYPTOPHAN DERIVATIVES INDOLE AND SEROTONIN ON VIRULENCE MODULATION OF ENTERIC PATHOGENS

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Bacterial pathogens employ different mechanisms to sense host and microbiota derived signals to regulate their virulence. Enterohemorrhagic *Escherichia coli* (EHEC) colonizes the colon by forming attaching and effacing (AE) lesions on enterocytes. EHEC employs a type III secretion system, encoded by locus of enterocyte effacement (LEE) pathogenicity island to form AE lesions. EHEC has a low infectious dose of 10-100 colony forming units. It senses different signals in the environment to tightly regulate its virulence gene expression. We showed that microbiota derived indole and host secreted serotonin are important signals that regulate virulence both in EHEC and in *Citrobacter rodentium*, a surrogate murine infection model of EHEC. We showed that in EHEC, tryptophan derivatives indole and serotonin are both sensed through membrane-bound histidine kinase CpxA. Upon sensing these signals, CpxA gets dephosphorylated and inactivates its response regulator CpxR. Inactivated CpxR can no longer activate virulence gene expression. We independently showed that indole and serotonin have the same effect and are sensed through CpxAR two-component system. The focus of our study is to integrate the effects of these signals in regulation of virulence gene expression in EHEC and gain a better understanding of indole and serotonin sensing. To investigate whether these signals act synergistically or compete with each other, we first optimized *in vitro* conditions in that both signals would regulate the LEE. We observed decreased LEE expression in response to either indole or serotonin. Transcription studies using qRT-PCR of the *LEE1-5* operons show that LEE transcription is decreased by indole or serotonin alone, but when both signals are present, they nullify each other's effect. The same phenotype is observed in the expression of the EspA and EspB protein, which are encoded within the *LEE4* operon. We also observed the antagonizing effect of serotonin and indole on *C. rodentium* pathogenesis *in vivo*. We altered indole levels in the gut using different genetically modified bacterial strains. Simultaneously, we manipulated serotonin levels by inhibiting the serotonin reuptake transporter (SERT) through genetic and pharmacological strategies. The findings of our study establish evidence for the presence of an important resistance mechanism by microbiota against enteric pathogens. Since many GI pathogens encode CpxA, it is possible to enhance the resistance against not only EHEC but against various infections through manipulating indole and serotonin levels by already established drugs (e.g., Prozac) and by manipulation of microbiota.

HOST DEMOGRAPHIC FACTORS ASSOCIATED WITH VAGINAL MICROBIOME COMPOSITION IN A MULTI-ETHNIC COHORT IN THE PACIFIC

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Purpose: Lactobacillus species dominate the vaginal microbiome and are important for maintaining vaginal health, but their specific taxonomy remains underexplored in populations throughout the Pacific. Using samples collected from women in Hawai'i's diverse communities, this project analyzes how host factors such as age, race, diet, birth control, stress and probiotic use influence Lactobacillus dominance.
Methods: Participants recruited via social media completed lifestyle/demographic questionnaires, and self-collected vaginal swabs. Returned swabs were subjected to 16s amplicon sequencing with V4 (515F/806R) primers and sequencing on the Illumia MiSeq platform. Raw demultiplexed paired-end FASTQ files were processed using the DADA2 pipeline (v1.28.0) in R. Taxonomy assignment at the genus level was performed using the SILVA database (v138.1). To achieve species-level resolution, we employed the SpeciateIT algorithm (vSpeciateDB models, 2024 release), Only assignments with non-zero posterior probability were retained. ASV abundance tables were converted into a VALENCIA-compatible format and each sample was assigned to a Community State Type (CST) and sub-CST based on nearest centroid similarity. Alpha diversity metrics (Observed richness, Shannon, Simpson) were calculated using estimate_richness() and compared across CSTs. Beta diversity was evaluated using Bray–Curtis distance metrics. Principal Coordinate Analysis (PCoA) was applied to visualize differences between microbial communities by CST. PERMANOVA (via adonis2) was used to test for statistical significance between groups.
Results: 47 participants completed the study. Average sequencing depth was 15,648 reads. Samples were rarified to 5,000 reads, resulting in 1945 OTUs. The majority of participants were Lactobacillus dominant. Demographic factors most impactful on Lactobacillus Dominance were age (post-menopause) and birth control type. Stress level, fermented food consumption, probiotic supplementation, and race and ethnicity did not have a significant effect on alpha or beta diversity, nor overall Lactobacillus Dominance.
Discussion: This preliminary study attempted to characterize the vaginal microbiome among a diverse cohort in Hawai'i. Further recruitment efforts are underway to broaden the socio-demographic exposures. Host immune responses will be compared among dysbiotic profiles vs those with Lactobacillus dominance.

CHARACTERIZATION OF THE ROLE OF PUTATIVE *AEROMONAS CAVIAE*-SPECIFIC VIRULENCE FACTOR, *FLGB*, IN VIRULENCE AND HOST-PATHOGEN INTERACTIONS

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Aeromonas caviae, Gram negative bacteria often present in water and soil, are emerging human pathogens associated with various infectious diseases. *A. caviae* present a public health risk due to emerging antimicrobial resistance, ability to infect immunocompromised and immunocompetent humans, and ubiquitous environmental presence. Despite recent studies demonstrating *A. caviae* is one of the most predominant *Aeromonas* species underlying human infections, *A. caviae* are understudied and no *A. caviae*-specific virulence factors associated with human disease have been identified. To identify *A. caviae*-specific putative virulence factors, we conducted comparative genomic analyses among clinical *Aeromonas* isolates to identify genes overrepresented in *A. caviae*. A variant of *flgB*, predicted to encode a polar flagellum rod protein, was significantly more abundant in *A. caviae* isolates. To examine the role of this *A. caviae*-specific *flgB* in virulence and host-pathogen interactions, we generated an *A. caviae flgB* deletion mutant and complementation constructs and assessed swimming motility, polar flagella production, adherence, proinflammatory cytokine production, and *in vivo* virulence. Motility and polar flagella assembly were abolished in the mutant and functionally rescued with complementation, demonstrating *flgB* is required for motility and polar flagella assembly in *A. caviae*. As it remains unknown where *A. caviae* infects in the human GI tract, we assessed host-pathogen interactions in HT-29 and Caco2 human intestinal cell lines, representative of the large and small intestine, respectively. Deletion of *flgB* significantly decreased bacterial adherence to HT-29, but not Caco2, cells. In both cell lines, IL-8, IL-13, IL-1β, and IL-6 production were significantly interrupted by *flgB* deletion; complementation partially rescued cyotkine production in HT-29 cells only. Differences between cell lines could indicate possible *A. caviae* tropism, warranting further study. Given the lack of relevant mammalian models for studying enteric pathogens *in vivo*, we utilized a *Galleria mellonella* larval survival model, where *flgB* deletion modestly attenuated virulence. Deletion of *flgB* altered aspects of virulence and host-pathogen interactions and this study provides a framework for identifying and characterizing additional *A. caviae*-specific virulence factors.

A DUAL REQUIREMENT FOR OUTER-MEMBRANE PROTEINS IN *HAEMOPHILUS INFLUENZAE*: IMMUNE EVASION AND ANTIBIOTIC RESISTANCE

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Nontypeable *Haemophilus influenzae* (NTHi) is a primary cause of exacerbated chronic obstructive pulmonary disease (COPD), the most common chronic lung disease and the sixth leading cause of death in the U.S. The macrolide clarithromycin, predominantly prescribed to treat NTHi-complicated COPD, is not effective against this bacterial pathogen due to intrinsic factors, such as the multidrug efflux pump AcrAB, and acquired mutations, such as nonsense mutations in the negative regulator AcrR, resulting in overexpression of AcrAB. To identify genes not previously implicated in clarithromycin resistance, we conducted a genome-wide-transposon insertion site sequencing screen in the presence of sub-inhibitory doses of clarithromycin and identified 33 clarithromycin resistance genes, including genes of lipooligosaccharide (LOS) synthesis and outer-membrane associated genes involved in maintaining the cell's permeability barrier. Because targeting gene products that are necessary for both clarithromycin resistance and infection in the lungs may limit antibiotic evasion and increase drug efficacy, we cross-referenced these genes with those required for infection in the murine lung model obtained from a prior transposon screening performed by our lab. Three genes required for both conditions, a heptosyltransferase (*orfH*), a putative outer-membrane protein (*omp26*), and a putative LOS modifying enzyme (HI0461), were evaluated in antibiotic susceptibility assays. Our results showed modest decreases in MIC in RdAW and Hi375 deletion mutants but a more substantial decrease in the NT127 deletion mutants. To determine whether deletion of *orfH* or *omp26* could override efflux-mediated resistance, we recreated the *acrR* mutation found in clarithromycin resistant clinical isolates in our model strain. Strikingly, deletion of *orfH* or *omp26* in the clarithromycin resistant strain completely abrogated the enhanced resistance conferred by the *acrR* null mutation. To investigate the mechanism behind the conferred sensitivity in the resistant background, we performed ethidium bromide accumulation assays. Deletion of *orfH* or *omp26* conferred increased accumulation in both wild type and *acrR* null backgrounds, while the resistant strain had the lowest accumulation. Finally, serum bactericidal assays were performed to examine the mechanism for the *in vivo* requirement for Omp26. Our data revealed that deletion of *omp26* resulted in a decrease in the survival rate compared to wild types in all strains except Hi375. Overall, our findings indicate that overexpression of the efflux pump is not sufficient to maintain resistance when the outer membrane or its components have been altered, and that both OrfH and Omp26 are required for immune evasion. Ultimately, these identified conserved gene products could be targeted to synergize with clarithromycin.

DETERMINING THE IMPACT OF IRON AVAILABILITY ON THE PHYSIOLOGY OF *MORGANELLA MORGANII*, AN EMERGING SUPERBUG

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Morganella morganii, a commensal of humans, fish, and other organisms, is prevalent in environmental, clinical, and agricultural settings. This opportunistic One Health pathogen is garnering attention for causing multidrug-resistant infections ranging from those of the urinary tract and post-operative wounds through meningitis and sepsis. *M. morganii* is a member of the SPICE organisms (*Serratia* spp. *Providencia*, Indole-positive *Proteus* spp. and others (e.g. *M. morganii*), *Citrobacter*, and *Enterobacter*)), a group of bacteria that initially appear drug-sensitive but express an inducible beta-lactamase which confers resistance to this class of antibiotics upon exposure. Despite its emergence as a potential superbug, vanishingly little is known about the factors contributing to *M. morganii* virulence, or indeed its basic biology, with less than 1,300 PubMed indexed papers published on the topic to-date. Iron availability, a key factor in the pathogenesis of almost all bacteria, is known to affect survival both *in vitro* and *in vivo*. Whilst iron starvation in bacteria is commonly investigated and typically results in upregulation of metal import systems and genes involved in the iron sparing response, iron intoxication is less well characterized. We observed that *M. morganii* forms a glucose-repressible unidentified black compound upon exposure to excess exogenous iron. Not only this, but robust transcriptional changes are induced upon iron exposure, including pathways putatively involved in iron detoxification and homeostatic regulation, as well as genes with no known function. Conversely, downregulated pathways include those associated with iron uptake. Given the phenotypic response of *M. morganii* to iron, transcriptional data, mass spectrometry, and targeted mutagenesis are being used to identify the black compound and elucidate mechanisms used by *M. morganii* to resist iron toxicity. Notably excess iron may mismetallate metalloproteins and potentiate the formation of reactive oxygen species, requiring detoxification to prevent damage. Further, not all clinical isolates of *M. morganii* produce this cryptic compound, and thus comparative genomics is being used to help identify contributing loci. Together we are working to further characterize the iron-responsive transcriptional profile of *M. morganii*, reveal the identity of the black substrate, and determine its role in iron homeostasis. Not only will this provide insights into the basic biology of *M. morganii*, it may also inform targetable pathways for future drug development.

ENTEROPATHOGENIC *ESCHERICHIA COLI* MANIPULATES THE HOST EXOCYST COMPLEX TO ENHANCE PEDESTAL FORMATION

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Enteropathogenic *Escherichia coli* (EPEC) produces plasma membrane pedestals that promote colonization of host cells. Critical for pedestal formation is EPEC's type III secretion system, which injects ~ 20 effector proteins into human cells. One of these effectors is Tir, which inserts into the host plasma membrane and stimulates the assembly of actin filaments essential for pedestal generation. To date, actin polymerization is the only host process known to contribute to pedestal formation. Here we report that EPEC co-opts the membrane trafficking pathway of polarized exocytosis, which acts together with actin polymerization to allow the efficient production and growth of pedestals. Polarized exocytosis is mediated by the exocyst — a human octameric complex that uses intracellular vesicles to expand the plasma membrane. We found that EPEC stimulated exocytosis at sites of pedestal formation in a manner dependent on the exocyst. The bacterial effector EspH recruited the exocyst and promoted exocytosis. Genetic inactivation of *espH* or RNA interference (RNAi)-induced depletion of exocyst components reduced both the frequency and size of pedestals and impaired colonization of host cells. Additional RNAi experiments indicated that the exocyst is dispensable for actin filament assembly in pedestals. Co-depletion of components of the exocyst and the Arp2/3 complex showed that exocytosis and actin polymerization make additive contributions to pedestal formation. Collectively, these results indicate that exocyst-mediated expansion of the plasma membrane acts together actin polymerization to optimize the generation of pedestals.

DECODING THE NASOPHARYNGEAL ECOSYSTEM: ENTEROTYPE SIGNATURES AND KEYSTONE MICROBIAL FEATURES AS PREDICTIVE BIOMARKERS FOR RESPIRATORY HEALTH

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The nasopharyngeal microbiome plays a critical role in respiratory health, yet comprehensive characterization across diverse populations remains limited. Through systematic meta-analysis of 7,790 nasopharyngeal samples from 28 studies, we characterized nine distinct nasopharyngeal enterotypes and their network topologies, providing a foundation for deeper investigation of microbiome-disease relationships.

Using these resources, we elucidate keystone microbial feature sets predictive of nasopharyngeal health status via a three-step framework: 1) feature space generation using compositional data analysis techniques strategically tailored to capture enterotype-specific microbial balances and network properties of the nasopharyngeal microbiome; 2) feature reduction to identify the most informative microbial signatures across health states and age groups; and 3) model generation and testing using diverse machine learning approaches. We validated these models using an independent cohort of >1,500 samples comprising healthy individuals and patients with SARS-CoV-2 infection, RSV infection, and acute otitis media.

Our refined models reveal significant age-dependent transitions between enterotypes, with microbiome diversity progressively increasing from infancy through adulthood along distinct developmental trajectories. Disease-specific perturbations demonstrate characteristic shifts in enterotype stability and diversity, particularly in respiratory infections and inflammatory conditions. A SARS-CoV-2 specific classification model achieved >90% accuracy in distinguishing infected from healthy individuals based solely on microbiome profiles. Validation in the independent cohort confirmed the stability of enterotype classifications, preservation of network topology across populations, and robust predictive performance of our models across diverse respiratory conditions.

This comprehensive analytical framework enhances understanding of nasopharyngeal microbiome dynamics across diverse age groups and disease states, establishing a foundation for future diagnostic tools and therapeutic interventions. The ability to detect subclinical microbiome perturbations offers promising applications for early disease detection, personalized medicine approaches, and novel therapeutic strategies targeting the nasopharyngeal microbiome.

BIFIDOBACTERIUM SPECIES MAY LIMIT CANDIDA EXPANSION IN PATIENTS WITH ACTIVE ULCERATIVE COLITIS

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Background: Candida species are increasingly associated with relapse and inflammation in UC. Bifidobacterium species have been found to be negatively correlated with Candida abundance, although whether Bifidobacterium species can limit Candida expansion in UC remains unknown.

Methods: We isolated and cultured Bifidobacterium and Candida species from the stool of actively relapsing UC patients and from lab-derived, commercially-available strains. Cell-free supernatants were prepared from microbial isolates. Twenty-four hour growth rates and viabilities of Candida species were evaluated following exposure to Bifidobacterium supernantants to assess their suppressive capacity. Targeted metabolomics was performed on supernatants to assess potential inhibitors of Candida growth.

Results: We prospectively recruited 6 patients for stool sampling during the active phase of UC. 9 distinct strains of Bifidobacterium species were successfully isolated from 5 patients. Candida glabrata was successfully isolated from 1 patient. Bifidobacterium CFSs demonstrated a 91.2% inhibition of C. albicans and 83.5% inhibition of C. glabrata. Bifidobacterium CFSs from clinical isolates were more effective at Candida suppression than ATCC-derived Bifidobacterium strains or Blautia producta strains. Supernatants that effectively suppressed Candida growth had significantly higher levels of isovalerate, 1-octanol, 1-decanol, isobutyrate, and capric acid.

Conclusion: Bifidobacterium species may provide colonization resistance against C. albicans in the UC gut. Medium chain fatty acids and branched-chain fatty acids may be associated with Candida suppression. Loss of Bifidobacterium strains may allow for C. albicans expansion which may contribute to inflammation and relapse.

COMPETITIVE CELL WALL MODIFICATIONS OF *STAPHYLOCOCCUS AUREUS* CONTROL RELEASE OF IMMUNOSTIMULATORY DNA DURING INFECTION

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Staphylococcus aureus (*S. aureus*) is a leading infectious cause of mortality worldwide. *S. aureus* causes persistent infections during which bacteria reduce toxin production and evade immune clearance. To define mechanisms of *S. aureus* immune evasion, we developed a macrophage infection model using noncytotoxic *S. aureus* strains to simulate host-pathogen interactions during persistent infection. Infection of murine bone marrow-derived macrophages (BMDMs) triggered release of IL-1β, a cytokine that coordinates immune responses to *S. aureus*. Consistent with prior studies, we found bacteria deficient for peptidoglycan-modifying enzyme O-acetyltransferase (OatA) triggered more IL-1β release than wild-type (WT) bacteria. To determine how OatA modulates immunity, we performed a bacterial genetic screen that revealed wall teichoic acid (WTA) glycosyltransferases TarM and TarS (TarMS) enhance IL-1β release. OatA-deficient *S. aureus* strains contained more WTA than WT strains, suggesting OatA blunts IL-1β release by blocking deposition of glycosylated WTA. IL-1β release is controlled by an immune complex called the inflammasome. To characterize host pathways impacted by cell wall modifications, we infected BMDMs deficient in inflammasome components and discovered IL-1β release requires cytosolic DNA sensor AIM2. OatA reduced and TarMS increased activation of another cytosolic DNA sensor, cyclic GMP-AMP synthase (cGAS), suggesting these modifications alter the release of bacterial DNA into infected cells. To test this hypothesis, we quantified free bacterial DNA within infected BMDMs and found OatA reduces whereas TarMS increase levels of free bacterial DNA. To characterize the cellular mechanism of AIM2 activation, we treated BMDMs with inhibitors of phagosome maturation and found that phagocytosis and phagosomal acidification are required for IL-1β release. Using cells producing fluorescent-tagged AIM2, we found *S. aureus* infection triggers formation of dense AIM2 puncta in close proximity to bacteria, suggesting release of bacterial DNA or escape of bacteria from phagosomes may directly trigger AIM2 activation. Finally, OatA reduced and TarMS increased IL-1β release during infection of primary human macrophages, suggesting cell wall modifications could play a role in immunity during human infections. Collectively, these data reveal competing roles for peptidoglycan O-acetylation and WTA glycosylation in immune responses to *S. aureus*, and suggest *S. aureus* regulates availability of its DNA within host cells to alter immune responses during noncytotoxic infections.

UNRAVELING METABOLIC REWIRING OF PATHOGENIC CANDIDA ALBICANS HYPHAE WITH UNTARGETED FLUXOMICS AND QUANTITATIVE PROTEOMICS

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Candida albicans is an opportunistic fungal pathogen that commonly resides in the oral cavity, vaginal tract, and gastrointestinal system. A critical aspect of Candida albicans pathogenicity is its ability to transition from a yeast form to a filamentous hyphal form. The transcriptional repressor nrg1 plays a key regulatory role in this morphological transition. The regulatory shift highlights two distinct mechanisms: transcriptional repression of nrg1 during hyphal initiation, and environmental regulation to inhibit nrg1 binding during maintenance. Since cellular metabolism is central to sensing and responding to environmental factors, we utilized nrg1-deleted strain of Candida albicans to investigate the metabolic processes that support hyphal maintenance. To investigate intracellular metabolism, we conducted time-series stable isotope tracing experiments using [U-13C6]glucose. Candida albicans wild-type and two mutant strains—locked in either yeast form (efg1 mutant) or hyphal form (nrg1 deleted)—were cultured in minimal media containing glucose as the sole carbon source. To address the limitations of conventional metabolomics approaches, we developed a computational pipeline for untargeted analysis of metabolic pathway utilization. One of the key findings from this untargeted analysis was that the hyphal-locked strain exhibited significantly slower amino acid biosynthesis from glucose compared to both the wild-type and yeast-locked strains. To explore the underlying mechanisms of this metabolic shift, we performed quantitative proteomics analysis. Our proteomics data revealed increased abundance of atg11 and decreased abundances of atg8 in the hyphal-locked strain compared to the wild-type. These changes suggest that selective autophagy may be upregulated in hyphal-locked cells to facilitate the degradation of intracellular components. This increase in autophagic activity may account for the reduced incorporation of glucose-derived carbons into amino acids, as autophagy likely contributes an alternative source to the intracellular amino acid pool in hyphal-locked cells.

BIFIDOBACTERIUM LONGUM BFD1R ATTENUATES COLLAGEN-INDUCED ARTHRITIS VIA TDCA-MEDIATED SUPPRESSION OF IL-17 PRODUCTION AND OSTEOCLASTOGENESIS

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Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by synovial inflammation and joint destruction. Emerging evidence suggests that gut microbiota play a pivotal role in modulating immune responses in RA.
Metabolite analysis was conducted on fecal samples derived from a collagen-induced arthritis model treated with BFD1R. Furthermore, the bile acid metabolite TDCA, which was upregulated following BFD1R treatment, was employed to assess its inhibitory effects on Th17 cell differentiation and osteoclastogenesis in vitro.
Here, we investigated the therapeutic potential of Bifidobacterium longum strain BFD1R in an experimental model of RA. Oral administration of BFD1R significantly alleviated joint inflammation and bone erosion in collagen-induced arthritis (CIA) mice. In the arthritis model treated with BFD1R, the bile acid metabolite TDCA was found to be upregulated. This increased TDCA directly suppressed Th17 polarization and reduced the expression of osteoclastogenic markers, including RANKL and cathepsin K, as confirmed by in vitro studies.
These findings highlight the potential of BFD1R as a novel microbiome therapy targeting the gut-joint axis in RA by modulating bile acid metabolism and IL-17-driven inflammatory pathways.

MUCIN PROMOTES COLONIZATION OF *STREPTOCOCCUS PNEUMONIAE* THROUGH PROLONGED SURVIVAL AND TRYPTOPHAN BIOSYNTHESIS

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Streptococcus pneumoniae (pneumococci) are Gram-positive cocci that generally exist as commensals on the human nasopharyngeal mucosa but are often characterized as pathogens due to its ability to disseminate into the inner ear and lower respiratory tract. Mucus serves as the protective barrier between the host epithelium and the environment and is postulated to serve as an environmental cue for the pneumococcus. Since mucin is abundant in pneumococcal environmental niches, we sought out the impact of mucin on various aspects of pneumococcal biology. We found that mucin, the most abundant macromolecule in mucus, delays pneumococcal autolysis and facilitates prolonged bacterial survival in stationary phase. Mucin effectively blocked autolysis via interference with the enzymatic activity of the primary autolysin, LytA. This inhibition of lytic activity resulted in decreased release of the pneumococcal cholesterol-dependent cytolysin, restricting host cell tissue damage. Global transcriptional analysis of pneumococci grown in mucin revealed altered expression of several key metabolic pathways including an upregulation of *de novo* tryptophan biosynthesis that resulted in the extracellular secretion of this essential amino acid. This *de novo* biosynthesis was found to be critical for both colonization and invasive disease, despite there being sufficient tryptophan to promote normal bacterial growth in serum. The data presented here suggest that environmental niches harboring mucins may promote pneumococcal colonization over invasive infection by restricting toxin release and prolonging bacterial survival.

DECODING HOW *STAPHYLOCOCCUS AUREUS* PEPTIDOGLYCAN MODIFICATIONS SHAPE THE HOST IMMUNE RESPONSE

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The cell envelope of *Staphylococcus aureus* aids in subverting host immune responses while providing protection against external stressors. It is mainly composed of peptidoglycan (PG), which gives shape and stability to the bacterium. PG comprises glycans containing disaccharide units of variable lengths, which are recognized by innate immune cells leading to pro-inflammatory cytokine production. However, we do not know how PG processing calibrates these responses. We initially tested if PG modifying enzymes known as glucosaminidases shape innate immune responses to *S. aureus* and found that the glucosaminidase, SagB, an enzyme that cleaves PG glycans, promoted the maturation and release of IL-1β by macrophages. SagB was also required for IL-1β production and inflammatory pathology in a skin and soft tissue infection model and virulence during systemic infection. Purified PG from a Δ*sagB* mutant elicited markedly reduced IL-1β from macrophages compared to wildtype PG and required both stem peptide and glycan components. In addition, SagB-mediated IL-1β production was independent of canonical pathways including caspase-mediated maturation and activation of the NLRP3 inflammasome. Nevertheless, IL-1β release and processing of pro-IL-1β to its mature form was defective after treatment of macrophages with a Δ*sagB* mutant, implying a previously unappreciated pathway of IL-1β maturation elicited by *S. aureus* glycans. We are currently determining the identity of the SagB-processed glycan that is responsible for inducing macrophage IL-1β production and the protease responsible for the maturation of pro-IL-1β. In addition, PG remodeling is important within clinical settings, as antibiotics that target cell wall synthesis are commonly used to treat infections. We tested if antibiotic treatment affected SagB-dependent IL-1β production by macrophages and found that treatment with sub-lethal concentrations of beta-lactams restored the ability of a Δ*sagB* mutant to promote IL-1β production. Because beta-lactams target penicillin-binding proteins (PBPs), we surmised that SagB-PBP crosstalk might be instrumental in calibrating IL-1β production. We generated a series of *sagB-pbp* double and triple mutants and found that SagB-PBP activities modified PG in several ways that promoted or dampened innate immune sensing. In summary, our work uncovers new insights into PG composition and innate immunity that promote *S. aureus* persistence during infection.

THE HIDDEN HIGHWAYS OF RESISTANCE: PLASMID-MEDIATED RESISTANCE IN *ENTEROBACTER*-ASSOCIATED MICROBIAL COMMUNITIES

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Gene flow in bacteria isn't confined to lineage, horizontal gene transfer (**HGT**) allows traits like antibiotic resistance to spread rapidly across species. This lateral exchange accelerates the dissemination of antimicrobial resistance (**AMR**), enabling resistance genes to thrive within diverse microbial communities. As these traits circulate, pathogens adapt beyond the reach of current therapeutics. If left unchecked, AMR is projected to cause 10 million deaths annually by 2050, disproportionately affecting vulnerable populations and straining global healthcare systems. Among the most concerning threats is the ***Enterobacter cloacae*** complex (Ecc), a group of seven closely related species that have emerged as multidrug-resistant opportunistic pathogens in hospital settings. Ecc members are particularly troubling due to their ability to acquire and transmit resistance genes via conjugative plasmids, serving as reservoirs of resistance within polymicrobial communities. Targeting these plasmids, and the mechanisms that govern their mobility, offers a promising strategy to disrupt or reverse resistance spread. To better understand the vulnerabilities in these dissemination pathways, we assembled a panel of over 100 clinical Ecc isolates. These isolates exhibited extensive and heterogeneous resistance profiles, with several harboring more than 15 antimicrobial resistance genes (ARGs) and collectively conferring resistance to nearly all major antibiotic classes. Whole-genome sequencing revealed that more than 60% of all identified ARGs were predicted to be plasmid-encoded, emphasizing the central role of mobile genetic elements in resistance persistence and transfer. Building on these findings, we are developing *in vivo* polymicrobial infection models to study plasmid mobility within complex communities under antibiotic pressure. By integrating functional assays and metagenomic analyses, our ongoing work aims to **uncover the ecological and genetic factors that drive plasmid-mediated resistance gene flow**. These insights will inform new strategies to mitigate resistance transmission and improve therapeutic outcomes in infection-prone clinical settings.

GROUP B *STREPTOCOCCUS* GLYCOLIPIDS PROTECT AGAINST IMMUNE CELL CLEARANCE DURING HOST-PATHOGEN INTERACTIONS

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Group B *Streptococcus* (GBS) is the leading cause of neonatal meningitis and frequently isolated from diabetic wounds. A critical site for host-pathogen interaction is the bacterial cellular membrane. Membrane lipids are important for cell division, protein localization, stress responses, and pathogenesis. GBS synthesizes three distinct glycolipids, the initial glycolipid (Glc-DAG) is synthesized by IagB and a GBSΔ*iagB* mutant lacks all glycolipids. We have previously evaluated the impact of GBS glycolipids in host-pathogen interactions and their role in inflammatory responses in multiple murine models of infection: i) a neonatal meningitis model and ii) adult diabetic wound model. GBSΔ*iagB* is cleared from the bloodstream of neonatal mice and the diabetic wound compared to WT GBS. In both niches, neutrophils play an important primary role in combating pathogens. Our investigations suggested that GBS glycolipids play an important role in protecting neutrophil clearance. *In vitro* interactions between neutrophils and GBS resulted in significantly reduced survival of GBSΔ*iagB* compared to GBS WT. Using primary human neutrophils in hyperglycemic conditions, we observe that both GBS WT and GBSΔ*iagB* induce primary and secondary degranulation. Delving into major mechanisms of neutrophil killing we identified GBS glycolipids are important for protecting GBS against cationic antimicrobial peptides and from reactive oxygen species. Additionally, we have identified GBS glycolipids are present in GBS membrane vesicles (MVs). The loss of glycolipids from MVs reduces the overall diversity of proteins in the MVs; however, MVs still elicit inflammation at the blood-brain barrier both *in vitro* and *in vivo*. Ongoing studies aim to further characterize the role of GBS glycolipids impact on immune cell clearance and GBS MVs induced dysfunction of the blood-brain barrier.

LATILACTOBACILLUS CURVATUS ALLEVIATES DSS-INDUCED COLITIS BY MODULATING TH17/TREG BALANCE AND GUT MICROBIOTA COMPOSITION

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Latilactobacillus curvatus has gained increasing attention as a beneficial commensal bacterium with potential anti-inflammatory properties. Although dysbiosis of gut microbiota is a key contributor to the pathogenesis of inflammatory bowel disease (IBD), the role of *L. curvatus* in the context of intestinal inflammation remains largely unexplored. We investigated the impact of orally administered *Latilactobacillus curvatus* on gut immune responses and microbial composition in mice with dextran sulfate sodium (DSS)-induced colitis.

In an in vitro spleen cell culture system, treatment with *L. curvatus* suppressed IL-17 production while promoting the expansion of regulatory T cells and increasing the secretion of IL-10. Furthermore, in colon fibroblast cultures that mimic fibrotic conditions, *L. curvatus* significantly reduced the expression of fibrosis-associated markers, including α-smooth muscle actin (α-SMA) and type I collagen. DSS-induced damage and the therapeutic effect of *Latilactobacillus curvatus* were investigated. Treatment with *L. curvatus* significantly attenuated the severity of DSS-induced colitis in vivo. In colon tissues, *L. curvatus* administration reduced the expression of proinflammatory cytokines such as interleukin IL-6, tumor necrosis factor-α, IL-1β, and IL-17, which are mainly produced by T helper 17 cells. Importantly, not only live bacteria but also *L. curvatus*-derived metabolites demonstrated therapeutic potential both in vitro and in vivo, effectively reducing inflammatory and fibrotic responses. The composition of the gut microbiome was significantly altered in *Latilactobacillus curvatus*-treated mice. Alpha diversity indices were restored, and the relative abundance of beneficial genera such as Bifidobacterium markedly increased following *L. curvatus* administration.

These findings suggest that *Latilactobacillus curvatus* may serve as a promising therapeutic candidate for inflammatory bowel disease by regulating the Th17/Treg balance, suppressing inflammatory cytokine expression, and inhibiting intestinal fibrosis.

MUCUS DIGESTION BY *RUMINOCOCCUS GNAVUS* INCREASED CYSTEINE ELABORATION AND ENHANCED H_2S PRODUCTION BY LF82

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Background: Mucus-digesting *Ruminococcus gnavus* (*Rg*), adherent-invasive *E. coli* (AIEC) and hydrogen sulfide (H_2S) are enriched in active Crohn's disease (CD) vs. healthy subjects. We identified a functional link between *Rg* and CD AIEC LF82 by showing that dual colonization of germ-free (GF) $IL10^{-/-}$ mice enhanced colitis and cecal H_2S production vs. LF82 alone. Mucus-derived monosaccharides including sialic acid (SA) enhance AIEC growth and L-cysteine is the primary substrate for AIEC H_2S production. Mucus, an initial mucosal defense against bacterial invasion, is composed with heavily glycosylated and sulfated glycoprotein. Cysteine disulfide bonds crosslink mucus polymers. RNAseq cysteine metabolic pathway analysis after H_2S exposure indicated *Rg* L-cysteine biosynthesis from sulfide and L-serine, a mucin amino acid. We explored mechanisms of *Rg* cysteine elaboration both *in vivo* and *in vitro* based on high H_2S production by dual colonization.
Hypothesis: *Rg* digests mucin to release L-serine and synthesizes L-cysteine to stimulate LF82 H_2S production, further inducing *Rg* cysteine synthesis to perpetuate LF82 H_2S production and colonic injury.
Methods: *In vivo*: We colonized wild type (WT) or $IL10^{-/-}$ GF mice with *R. gnavus* for 5d and harvested cecal samples to measure SA and L-cysteine concentrations by liquid chromatography-tandem mass spectrometry (LC-MS/MS). *In vitro*: LF82 was cultured in GF or *Rg*-colonized cecal samples to measure H_2S production. *Rg* cultured in M9 minimal medium +/- H_2S 1mM for 24 hrs underwent RNA sequencing.
Results: SA, L-cysteine and LF82 H_2S production were higher in *Rg*-colonized cecal samples. Strong correlations existed between SA and luminal *Rg* bacterial number ($r= 0.79$, $p<0.001$), and between luminal L-cysteine and *in vitro* normalized H_2S ($r= 0.72$, $p<0.001$). H_2S exposure upregulated expression of *Rg* genes related to L-cysteine biosynthesis from L-serine.
Conclusions: *Rg* colonization of ex-GF mice increased luminal sialic acid, L-cysteine, and LF82 H_2S release. High H_2S exposure upregulated the *Rg* L-cysteine biosynthesis pathway. We postulate that *Rg* synthesizes L-cysteine to stimulate LF82 H_2S production that degrades mucus crosslinking, injures epithelial cells and augments *Rg* synthesis of L-cysteine, initiating a vicious cycle to potentiate colitis. Adding L-serine +/- H_2S to *Rg* culture to confirm increased *Rg*-cysteine synthesis and filtered medium to measure LF82 H_2S production is pending. Elucidating mechanisms underpinning AIEC and *Rg* synergy may reveal novel approaches to treat microbially driven inflammation in CD.

MULTIPLE ACCESSORY PROTEINS MODULATE PHOSPHATE HOMEOSTASIS AND CONTRIBUTE TO PATHOGENESIS IN *STAPHYLOCOCCUS AUREUS*

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Phosphate is indispensable for life and proper regulation of phosphate homeostasis is necessary for microbial survival. The molecular details of bacterial phosphate homeostasis have primarily been investigated in *Escherichia coli*. However, genomic analysis suggests that microbial control of phosphate homeostasis is more complex, with most bacteria having multiple homologs of the single *E. coli* accessory regulatory protein PhoU. Despite the essentiality of phosphate homeostasis, the impact of these additional regulators on this process and microbial pathogenesis is unknown. One of the organisms that possesses a full complement of accessory proteins is the human pathogen *Staphylococcus aureus*. Leveraging *S. aureus* revealed that all three accessory proteins PitR, PhoU, and a PhoU-domain associated with the phosphate importer NptA influence phosphate homeostasis. These investigations also demonstrated that the three homologs control phosphate homeostasis in a hierarchical manner. Notably, PitR, absent in *E. coli*, is the dominant regulator of phosphate in *S. aureus* with PhoU subordinate to it and the PhoU-domain of NptA in standard culture conditions. However, the hierarchy is modulated by the environmental conditions, leading both PitR and PhoU to independently contribute to the ability of *S. aureus* to cause infection. These observations uncovered that the expanded repertoire of accessory proteins enables microbes to maintain phosphate homeostasis in diverse environmental conditions, including those encountered by pathogens during infection.

THE COPPER TRANSPORTER CERULOPLASMIN IS DEGRADED BY THE PNEUMOCOCCAL NEURAMINIDASE NANA AND IMPACTS VIRULENCE IN AN ALLELE-DEPENDENT MANNER

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Streptococcus pneumoniae, or pneumococcus, is the primary cause of community-acquired pneumonia, and also causes meningitis, otitis media, and septicemia. The innate immune response uses various acute-phase proteins (APP) to aid in bacterial clearance through multiple mechanisms. The pneumococcus can bind various APPs to reduce the effectiveness of the innate immune response, but such binding can also provide other benefits to the bacteria. Ceruloplasmin, Cp, is a major copper transport protein and a serum ferroxidase. Cp also reduces reactive oxygen species formation in the tissue through oxidizing Fe (II) into Fe (III). This study aims to investigate how genotypic variations in pneumococcal strains alter the binding to Cp and impact host-pathogen interaction. A major virulence factor of this bacterium is the surface-anchored neuraminidase NanA, which cleaves host sialic acid and facilitates bacterial colonization and immune evasion. Despite its recognized role in pathogenesis, the molecular mechanisms by which NanA influences host responses remain incompletely understood. This study aims to examine how genotypic variations in pneumococcal NanA alter the binding to serum protein Cp and impact host-pathogen interaction, and determine whether targeting NanA therapeutically improves disease outcome. A library of 96 pneumococcal strains was sequenced, and high-throughput flow cytometry was used to study the binding of Cp. The effect of NanA on host-pathogen interaction was determined by static biofilm assays and murine colonization models. Protein degradation was assessed via SDS-PAGE and Coomassie staining. Viral neuraminidase inhibitor oseltamivir was used to determine its effect on pneumococcal sialidase activity. NanA was found to both reduce Cp binding and cause its degradation. The impact of different *nanA* alleles on biofilm formation varies, and allele-dependent differences have also been observed in colonization efficiency and bacterial load within the nasal, heart, and lung tissues. Additionally, oseltamivir has been shown to effectively inhibit NanA activity. This is the first described mechanism of ceruloplasmin interaction in the pneumococcus and highlights allele-dependent variation in its contribution to pneumococcal virulence. Targeting NanA activity with inhibitors such as oseltamivir may offer a novel therapeutic strategy to mitigate host tissue damage and improve outcomes in pneumococcal infections.

MAST CELL-SPECIFIC GPCR MRGPRB2 REGULATES BLADDER IMMUNITY DURING UTI

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Urinary tract infections (UTIs), primarily caused by uropathogenic *Escherichia coli* (UPEC), affect millions of women annually. During UTI, the bladder mounts an immune response to clear the pathogen by recruiting various immune cells. Mast cells are tissue-resident granulocytes known for their involvement in type 2 immunity and allergy but also serve as sentinels against invading pathogens. The mast cell-specific G protein-coupled receptor (GPCR) Mrgprb2, the mouse homolog of human MRGPRX2, has been shown to detect quorum-sensing peptides from bacteria and recruit neutrophils to fight against skin, lung and peritoneal infections, but its role in bladder immunity remains unknown. To investigate the role of Mrgprb2 during UTI, we infected C57BL/6J wild-type (WT) and *Mrgprb2*-KO mice with 10^7 CFUs of UPEC strain UTI89 for 24 hours and then collected the bladders for CFU enumeration, bulk-RNA sequencing, and histology. Our results show that *Mrgprb2*-KO mice cleared out UPEC from the bladder more effectively than WT mice. RNA-seq results reveal that the mast cell receptor Mrgprb2 regulates bladder immunity during UTI by recruiting immune cells and regulating bladder exfoliation. Our results point to mast cells and Mrgprb2/MRGPRX2 as potential therapeutic targets to improve patient outcomes during UTI.

ESTROGEN SIGNALING CONTRIBUTES TO GROUP B STREPTOCOCCAL DISRUPTION OF THE BLOOD-BRAIN BARRIER

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Bacterial meningitis is a serious life threatening infection of the central nervous system (CNS) that occurs when bacteria are able to interact with and penetrate the blood-brain barrier (BBB). The BBB is primarily comprised of highly specialized brain endothelial cells (BECs) that possess properties that allow for the import of nutrients while excluding toxins, drugs, and pathogens. BECs, when compared to peripheral endothelium, have more complex tight junctions, tightly regulated rates of endocytosis, and highly active multi-drug efflux transporters. Group B Streptococcus (GBS) is the leading cause of neonatal meningitis and presently there is no vaccine available. Using highly engineered induced pluripotent stem-cell technologies to model the BBB, we have previously shown that GBS is able to disrupt these key attributes of BECs however the mechanism behind this disruption is unknown. Here we show BECs activate an estrogen signaling program during GBS infection through transcriptomic and kinomic analysis suggesting that estrogen signaling activated by GBS may contribute to BBB disruption. Manipulation of estrogen signaling through inhibitors leads to less GBS invasion of BECs, improved efflux transport, and restricted endocytosis rates improving canonical BBB properties. Results were confirmed in vivo as mice treated with estrogen inhibitors had fewer bacterial counts present in the brain. Conversely, activation of estrogen signaling by beta-estradiol leads to greater bacterial invasion, and inhibition of transporters while increasing endocytosis to levels similar to GBS infection alone. Again, results were confirmed in vivo as mice treated with beta-estradiol had higher bacterial counts in the brain. Importantly, infected cells treated with the estrogen signaling inhibitor fulvestrant demonstrated a kinomic pattern more similar to uninfected BECs suggesting that estrogen signaling is the key to the host signaling pattern response. Here for the first time, we are able to demonstrate that estrogen signaling contributes to GBS mediated BBB dysfunction.

STRUCTURAL BASIS OF GLYCOCHOLATE-MEDIATED CONFORMATIONAL CHANGES IN THE TOXS PERIPLASMIC DOMAIN AND A MODEL FOR TOXRS ACTIVATION

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ToxRS and ToxRS-Like protein systems are a group of co-component transmembrane transcriptional regulators that regulate transcription in response to periplasmic signals and act as stress sensors in diverse bacterial species. For example, in *Photobacterium profundum*, a ToxRS-Like system mediates adaptation to high hydrostatic pressure, and in *Aliivibrio fischeri*, one contributes to adaptation and colonization. These two-protein systems are thought to work by the binding partner sensing environmental stimuli and relaying the signals through periplasmic domains to the membrane-embedded DNA-binding transcription factor. Additionally, several human pathogens have co-opted these systems to regulate pathogenesis and virulence gene expression. The ToxRS system is conserved throughout the *Vibrio* family, including *V. parahaemolyticus*, where it also regulates virulence. In this system, ToxR is a transmembrane transcription factor, and ToxS serves as the binding partner that is thought to sense stimuli. In *Vibrio cholerae* and *V. parahaemolyticus*, ToxR activity is stimulated by several factors including bile salts, which are bactericidal cholesterol metabolites. Enterotoxigenic bacteria, such as *V. cholerae* and *V. parahaemolyticus*, sense bile salts as a stress response for survival, and have co-opted this system to regulate virulence gene expression. To understand how ToxS senses bile salts, we solved the structures of the *V. parahaemolyticus* ToxS periplasmic domain in the absence and presence of the bile salt glycocholate. The apo form adopts an 8-stranded broken β-barrel with a central α-helix and is structurally homologous to a broad class of periplasmic chaperone proteins. Interestingly, the glycocholate-bound structure forms a dimer with the last β-strand domain-swapped. Modelling two copies of the ToxR periplasmic domain onto the glycocholate-induced ToxS dimer suggests a mechanism for how ToxRS and other ToxRS-Like proteins are activated.

SEROTYPE-INDEPENDENT CAPSULE PROPERTIES IMPACT *KLEBSIELLA PNEUMONIAE* HOST INTERACTIONS

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Klebsiella pneumoniae bacteremia is a significant public health burden with a 26% mortality rate which increases when the infecting isolate is multi-drug resistant. *K. pneumoniae* is the primary cause of neonatal sepsis in low- and middle-income countries. An important virulence factor of *K. pneumoniae* is the capsule, the protective polysaccharide coat that surrounds its outer membrane and is made up of individual capsular polysaccharide (CPS) chains. Capsule can differ in abundance, attachment, and length of the individual capsular polysaccharide chains. Long, uniform CPS chains are associated with a high level of mucoidy. Typically, mucoid CPS is produced by the hypervirulent *K. pneumoniae* (hvKp) pathotype, which is associated with invasive community-acquired infections. This is in contrast to the classical *K. pneumoniae* (cKp) pathotype, which tends to be non-mucoid and is associated with nosocomial infections and multi-drug resistance. There are over 80 serotypes of *K. pneumoniae* capsule. Capsule swap experiments have begun to reveal the effect of serotype on virulence and immune interactions. Clinically, the K2 capsule serotype is a common hvKp serotype associated with neonatal sepsis cases. However, cKp can also produce K2 capsule. It is unknown how cKp and hvKp strains differ in a bloodstream infection when producing the same capsule serotype. To fill this gap in knowledge, we characterized the surface properties of K2 serotype cKp and hvKp bloodstream infection isolates, then tested the fitness of these strains in bloodstream infection-related in vitro and in vivo assays. We measured the survival of these strains in human serum and blood, attachment of monocytes, and dissemination patterns from the blood in a murine model of bloodstream infection. We found that K2 hvKp strains have higher mucoidy and higher bacterial burden in the liver and spleen after dissemination from the blood compared to K2 cKp strains. Unexpectedly, we found no difference in capsule abundance between the strains. Understanding how K2 cKp and hvKp strains differ in pathogenic potential will give us further insight on how *K. pneumoniae* capsule properties influence bloodstream infection pathogenesis.

LITHOCHOLIC ACID INDUCES T3SS-DEPENDENT AGGREGATION OF *SHIGELLA FLEXNERI* WITH IMPLICATIONS FOR CELL INVASION

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Shigella flexneri (*Shigella*) causes bacillary dysentery, a disease that ranks as the second leading cause of diarrheal death globally. Following fecal-oral transmission, *Shigella* invades colonic epithelial cells and spreads intercellularly, causing epithelial damage and bloody diarrhea. While invasion and spread have been extensively studied, the extracellular phase that precedes invasion remains poorly understood. Notably, the low infectious dose (10-100 bacteria) and recent *in vivo* studies suggest that *Shigella* proliferates extracellularly in the colonic lumen as multicellular aggregates. Given that bacterial aggregation often contributes to pathogenesis, we investigated how *Shigella* aggregates form and whether they remain virulent. Previous work, including our own, showed that 2.5 mM deoxycholic acid (DCA) promotes *Shigella* aggregation *in vitro*, implicating bile acids as physiological cues. Here, we demonstrate that lithocholic acid (LCA), another abundant colonic bile acid, is significantly more potent, inducing robust aggregation at concentrations as low as 0.05 mM. Comparative genetic analysis revealed two distinct aggregation mechanisms: LCA-induced aggregation requires the virulence plasmid-encoded type III secretion system (T3SS) and its tip protein IpaD, whereas DCA-induced aggregation is primarily dependent on chromosomal factors and is IpaD-independent. To assess infectivity, we exposed HT-29 cell monolayers to aggregates formed in 0.05 mM LCA. Wide-field fluorescence microscopy confirmed that these aggregates can invade and spread within epithelial monolayers. Confocal microscopy showed that LCA-induced aggregates enter cells as intact clusters, rather than dispersing into single bacteria, suggesting a distinct invasion mode from planktonic *Shigella*. Collectively, these findings identify LCA as a potent extracellular signal that organizes *Shigella* into invasion-competent aggregates. Ongoing work aims to elucidate the role of bile acid-induced *Shigella* aggregates in disease pathogenesis.

A *STAPHYLOCOCCUS AUREUS* TOXIN ACCELERATES EPITHELIAL STEM CELL–MEDIATED WOUND REPAIR

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Cytolytic toxins are ancient protective mechanisms found in bacteria and higher organisms such as sea anemones, box jellyfish, earthworms, and plants. While typically known for their destructive functions, our data suggest that a microbial cytolytic toxin can promote healing in physiological wounds. In a human study, we observed that acute wounds harbored both commensals and pathobionts. Using a common mouse model of wound healing, we screened human wound isolates and were surprised to find that only *S. aureus* isolates accelerated wound closure. Through advanced bacterial genetics and purified virulence factor screening, we identified the active factor as a secreted toxin. This toxin is known to engage immune and endothelial cells, form cytolytic pores, and induce cell death. However, in skin epithelium, the toxin promotes repair through a cell death-independent mechanism. We are currently working to identify the toxin receptor on skin epithelial cells using membrane receptor screening and mass spectrometry. Additionally, we are characterizing its downstream molecular mechanism through bulk RNA sequencing and spatial transcriptomics at the wound edge. This research challenges the conventional view of bacterial toxins as purely destructive agents, offering insights into the interplay between microbes and host tissue repair mechanisms, and opening new avenues for wound healing therapies.

EFG1-REGULATED NETWORKS CONTROL CARBON AND NITROGEN UTILIZATION IN *CANDIDA ALBICANS*

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Candida albicans relies not only on morphogenic flexibility, mediated by key transcriptional regulators like Efg1, but also on metabolic adaptability to thrive within diverse host environments. While Efg1's role in filamentation, white-opaque switching, biofilm formation, and pathogenic interactions is well established, less is known about its contribution to metabolic regulation during nutrient uptake. Here, we investigate the consequences of *EFG1* deletion on *C. albicans* metabolism. **Phenotypic growth analysis:** The *efg1* null mutant showed growth defects when cultured on multiple nutrient sources as compared to the wild-type strain (WT; SC5314). In particular, WT cells exhibited biphasic growth on modified GAM (mGAM) media, whereas *efg1* null mutants were defective in growth during the second phase. **Metabolomic profiling:** Analysis of intracellular metabolites during the second growth phase revealed compromised uptake of amino acids and decreased hexose and purine/pyrimidine metabolism in the mutant. **Transcriptomics:** RNA sequencing showed significant repression of genes involved in the tricarboxylic acid cycle, glycolysis/gluconeogenesis, sugar metabolism, pyruvate metabolism, and the pentose phosphate pathway in the *efg1* null mutant. Genes associated with the uptake and metabolism of certain amino acids, often linked to virulence, were also downregulated in the mutant. **Phenotype validation:** Biolog growth assays across a range of carbon and nitrogen sources corroborated our multi-omics findings and confirmed the reduced metabolic capability of the *efg1* null strain. **Respiration defects:** Basal respiration and oxygen consumption rates showed compromised mitochondrial function and respiratory efficiency in the mutant. Together, our data show that Efg1 is a key metabolic regulator in *C. albicans*. Its deletion compromises central carbon metabolism, amino acid uptake and metabolism, and mitochondrial respiration. These processes are essential for nutrient adaptation and host persistence. Efg1, therefore, plays a multifaceted role in *C. albicans*, beyond its established roles in morphogenesis and biofilm development.

STRUCTURAL ELEMENTS REQUIRED FOR STABILITY OF BACTERIAL THIOL-DISULFIDE OXIDOREDUCTASES REVEALS A PROMISING TARGET AGAINST BACTERIAL INFECTION

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Bacterial thiol-disulfide oxidoreductase enzymes are a conserved protein family that catalyzes disulfide bond formation and loss of this protein in numerous pathogens significantly or completely diminishes their virulence. In search of a redox partner for the membrane-bound thiol-disulfide oxidoreductase MdbA in *Corynebacterium diphtheriae*, we serendipitously found that genetic disruption of TlpA- family protein disulfide reductase and cytochrome c biogenesis-like (CcdA) proteins led to a point mutation and a single base insertion altering the β1-β2 loop and α7 helix of MdbA, respectively. Although recreation of individual mutations in MdbA did not cause any significant defects, the combined mutations abrogated MdbA expression, accompanied with complete elimination of pilus assembly, the phenotypes that were similarly observed in the double mutant lacking *tlpA* and *ccdA*. Equivalent mutations generated in the thiol-disulfide oxidoreductase MdbA of *Actinomyces oris*, altering α7 helix of *A. oris* MdbA, caused its degradation and pilus assembly defects, concomitant of severe deficiencies in pilus-mediated polymicrobial interaction. Strikingly, disruption of the α7 helix in the *Escherichia coli* thiol-disulfide oxidoreductase DsbA also abolished the expression of this enzyme and its ability to rescue the pilus assembly defect of the *C. diphtheriae* mdbA mutant. The results support that the α7 helix is a conserved feature of bacterial thiol-disulfide oxidoreductase enzymes that is critical for their stability. Importantly this region of DsbA might be a novel target for antivirulence strategy. With the growing concern of reemerging pathogens due to antimicrobial resistance, small molecule inhibitors targeting α7 helix could selectivity impairs the bacterial pathogenicity without exerting selective pressure, provides a alternative approach to traditional antibiotics.

ESTABLISHING INTERCLADE GENE ESSENTIALITY WITHIN THE C. AURIS PANGENOME

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The emerging pathogen *Candida auris* is a critical public health threat that forms tenacious biofilms on both patient skin and hospital infrastructure, encouraging the continual spread of infection. There are currently 5 defined clades of this pathogen, which are phylogeographically distinct and feature several chromosomal rearrangements and thousands of nuclear single nucleotide polymorphisms between each group, though all exhibit superbug-like resistance to frontline antifungals. This high degree of multidrug resistance and nosocomial persistence highlights an urgent need to identify new therapeutic targets. This first requires a foundational understanding of gene essentiality in *C. auris*, especially with respect to its highly variable genetic content between clades. To identify core and accessory genes within *C. auris* variants, we performed a thorough pangenome analysis, using multiple available tools to ensure accuracy in annotation and orthogroup identification. Additionally, we identify genic regions of essentiality within individual *C. auris* clade representatives through insertion mutagenesis using an antibiotic cassette marker, followed by a transposon sequencing strategy. Pairing our pangenomics approach with essentiality mapping allows us to accurately select essential core genes that are either unique to *C. auris*, indicating promising species-specific targets, or are interspecies-essential orthologs that make ideal targets for broad-spectrum antifungals. This same approach allows us to classify therapeutic targets that are only essential in select genetic *C. auris* backgrounds but are tolerated in other clades, due to genetic background effects. We anticipate this study will set the necessary groundwork for future antifungal development.

IDENTIFICATION AND IMMUNOLOGICAL VALIDATION OF MHC-II–PRESENTED PEPTIDES FROM *FRANCISELLA TULARENSIS*

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Francisella tularensis is a Gram-negative, facultatively intracellular, and highly virulent bacterium that causes the serious zoonotic disease tularemia. In laboratory settings, the attenuated strain *F. tularensis* LVS is commonly used as a model organism due to its ability to induce a strong and protective immune response in mice. At sublethal doses, both innate and adaptive immune components are activated and cooperate to promote long-term survival and bacterial clearance. Among these, T cell-mediated mechanisms play a critical role. However, the specific antigens that serve as T cell epitopes during *F. tularensis* infection remain poorly characterized. This study builds on previous immunopeptidomic experiments that identified bacterial peptides presented on MHC-II molecules during *in vitro* infection of murine bone marrow-derived dendritic cells (BMDCs) with *F. tularensis* LVS. To evaluate the biological relevance of these candidate epitopes, their immunogenicity was subsequently assessed and validated in an *in vivo* model of infection. The findings could contribute to a deeper understanding of host immune responses and may support the development of tools for immune monitoring and the identification of protective antigens. Epitope identification was performed by stimulating whole-cell suspensions or isolated T cells obtained from the spleens, lymph nodes, or peripheral blood of *F. tularensis* LVS-immunized mice. Cytokine responses, including IFN-γ, TNF-α, and IL-2 production, were measured using the ELISpot assay following stimulation with candidate peptides. Based on these analyses, we report the identification and *in vivo* validation of ten novel *F. tularensis* epitopes, including both major and minor antigens capable of eliciting antigen-specific T cell responses.

UNDERSTANDING HOW ARAC REGULATION IMPACTS ENTERIC-BACTERIAL PATHOGENESIS USING REGA AS A MODEL SYSTEM

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Diarrheal illnesses are the second leading cause of death worldwide in children under five. Of these, bacterial infections caused by pathogenic strains of Escherichia coli (E. coli) (i.e., enterotoxigenic E. coli (ETEC), and enteroaggregative E. coli (EAEC)) significantly contribute to mortality and morbidity. AraC/XylS superfamily virulence regulatory proteins (AraC-VRs) are present in these and many other enteric gram-negative bacterial pathogens, controlling the expression of virulence genes such as toxins and attachment factors. Recent research suggests these virulence regulators are inhibited upon binding fatty acids, which, combined with their essential role in pathology and high structural homology, makes AraC-VRs attractive targets for antivirulence drugs. To explore this possibility and investigate the mechanism of inhibition by small molecule binding, we are investigating the AraC-VR RegA in Citrobacter rodentium, as it is a common model for studying E. coli infections in mice. RegA is homologous to Rns in ETEC, and ToxT in Vibrio cholerae, both of whose crystal structures have been solved with fatty acids bound to a structurally conserved N-terminal binding pocket. Preliminary results indicate that, despite low sequence homology in the N-terminal binding pocket, RegA is more strongly inhibited in vivo when compared to Rns by both fatty acids and regacin, a small molecule inhibitor. We hypothesize that, like its homologs, RegA binds to small molecules in its N-terminal binding domain, and that structural differences between RegA and other AraC-VRs are responsible for its increased sensitivity to ligand binding.
To address this, we solved the crystal structure of apo RegA and saw variation within the N-terminal binding pocket when compared to Rns. We also solved the regacin-bound RegA crystal structure and have investigated binding interactions through structural analysis and mutagenesis. Preliminary analysis has uncovered residues that, when altered, cause RegA to respond differently to ligand binding. In conclusion, we have solved the structures of both apo and ligand-bound RegA, characterized its binding pocket, and identified key residues that contribute to ligand-induced inhibition.

AMMONIA-PRODUCING GUT MICROBES ARE INCREASED IN PATIENTS WITH GASTRIC CANCER, AND AMMONIA INDUCES TIM3 EXPRESSION AND EXHAUSTION OF PATIENT T CELLS

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Objective: Dysbiosis of the gut microbiome has been associated with gastric carcinogenesis. In this study, we aimed to identify bacterial biomarkers that characterize the microbiome of gastric cancer (GC) patients and to investigate the contribution of microbial metabolites to gastric cancer progression. We found that ammonia-producing bacteria were enriched in the microbiome of GC patients and assessed the effects of ammonia on functions of T cells from GC patients.

Methods: LEfSe analysis was performed to identify microbial biomarkers that distinguish GC patients from healthy controls (HCs). We investigated the effects of ammonia on the expression of inflammatory cytokines, exhaustion markers, and cell death in T cells derived from GC patients using flow cytometry and ELISA. Its effect on mitochondrial function in these T cells was evaluated using JC-1 and MitoSOX Red staining. Additionally, the effects of ammonia on cell death and mitochondrial dysfunction were assessed in the Jurkat lymphoma cell line.

Results: LEfSe analysis identified bacterial markers that differentiate HCs from GC patients. In the HC, short-chain fatty acid (SCFA)-producing bacteria were enriched, and SCFAs are known to have anti-tumor effects. In the GC patients, ammonia-producing bacteria, including Streptococcus salivarius and Bacteroides vulgatus, were enriched. We investigated the adverse effects of ammonia on T cell function in GC patients and found that it reduced the expression of IFN-γ, TNF-α, and Granzyme B, while increasing TIM-3 expression in IFN-γ^- T cells. Additionally, ammonia induced cell death and increased mitochondrial membrane potential and ROS production in both patient-derived T cells and Jurkat cells.

Conclusion: Biomarkers differentiating healthy controls (HCs) and gastric cancer (GC) patients were identified. Among them, ammonia-producing bacteria were enriched in the GC group. Ammonia induced dysfunction and cell death in T cells derived from GC patients, and also caused mitochondrial dysfunction.

PROGRAMMED BACTERIAL LYSIS IN VIVO ALTERS HOST IMMUNE RESPONSES AND MICROBIAL PATHOGENESIS

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Vibrio cholerae kills intestinal bacteria in a process that enhances mucosal host immune responses and its own pathogenicity. Here we use CRISPRi to knock-down *in vivo* expression of essential genes in *V. cholerae* to test if its lysis influences pathogenicity and colonization of a bystander strain. Our results indicate that blocking of bacterial cell wall biogenesis with CRISPRi at different steps leads to divergent mucosal immune responses and disease outcomes. We identified two peptidoglycan-derived metabolites that show these divergent properties when perorally co-administered with *V. cholerae*. These data suggest that small intestinal exposure to different bacterial cell wall metabolites can differentially modulate bacterial pathogenesis and the host mucosal immune responses. This synthetic biological approach of engineering programmed bacterial lysis *in vivo* may have applications for the control of neoplasm and other diseases of immune dis-regulation.

PROTEIN CITRULLINATION BY *PSEUDOMONAS AERUGINOSA* PYOCYANIN DYSREGULATES AIRWAY MUCUS HOMEOSTASIS

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Pseudomonas aeruginosa (PA) is a clinically significant opportunistic pathogen that colonizes chronically diseased lungs such as cystic fibrosis and chronic obstructive pulmonary disease. Pyocyanin (PCN), a potent redox-active secondary metabolite and major virulence factor secreted profusely by PA that disrupts airway epithelial function and mucus homeostasis. However, the molecular mechanisms by which PCN drives mucus overexpression remain incompletely defined. This study investigates the role of PCN-induced protein citrullination in modulating airway goblet cell differentiation and proliferation, mucus production, and its relevance in the mucus pathogenesis of diseased lungs. Human bronchial epithelial cells (HBECs) exposed to clinically relevant PCN concentrations (5 μg/ml) showed increased intracellular calcium (Ca^{2+}), leading to the activation of Ca^{2+}dependent peptidylarginine deiminases (PADs) that convert arginine residues into citrulline. Enzymatic assays confirmed elevated PAD activity post-PCN treatment. Proteomic analysis identified 71 citrullinated proteins, including several potentially involved in cell differentiation and proliferation in the lung. Among these, we focused on Docking Protein 2 (DOK2), an adaptor protein regulating Ras signaling via interaction with Ras GTPase-activating protein (RasGAP or RASA1). Western blotting and immunofluorescence microscopy revealed that citrullinated DOK2 exhibits enhanced binding to p-EGFR and RASA1. This interaction impairs RASA1 function, diminishing its ability to inhibit Ras activity and resulting in sustained Ras activation. Persistent EGFR/Ras led to goblet cell hyperplasia and metaplasia, resulting in significant mucus dysregulation in HBEC cultured at the air-liquid interface (ALI) and in mouse lungs chronically exposed to PCN. Notably, treatment with the pan-PAD inhibitor BB-Cl-Amidine significantly reduced mucus secretion in both HBEC ALI cultures and in mice chronically exposed to PCN, validating the physiological relevance of PCN-induced citrullination. These findings establish a novel mechanistic link between PCN-induced citrullination, particularly of DOK2, which disrupts Ras signaling, and promotes goblet cell hyperplasia, metaplasia, and excessive mucus production. This study reveals how PA manipulates host cell signaling to promote disease pathology and underscores the therapeutic potential of targeting the p–EGFR–DOK2–RASA1 axis to mitigate mucus hypersecretion and airway obstruction in patients with diseased lungs predisposed to PA infection. Future efforts will explore other novel mechanisms by which PCN-mediated citrullination alters epithelial biology and identify potential therapeutic targets to combat mucosal dysfunction in diseased airways.

POPULATION DYNAMICS AND PATTERNS OF ANTIMICROBIAL RESISTANCE IN *SALMONELLA* TYPHI FROM HUMAN SOURCES IN NEW YORK STATE, 2018 – 2023

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The human-adapted foodborne pathogen *Salmonella enterica* serovar Typhi is the causative agent of enteric fever, a life-threatening and systemic infection that disproportionately affects low- and middle-income countries. Compounding on this significant public health crisis is the continued development of multidrug resistance, which limits the effectiveness of treatment options and worsens disease outcomes. Although *Salmonella* Typhi (STy) incidence is relatively low in the United States, with cases of infection generally associated with travel, routine surveillance of isolates recovered from clinical settings is essential for monitoring the extent of antimicrobial resistance, pathogen risk status, and lineage dynamics of STy. In this study, we analyzed 110 STy genomes isolated from human sources across New York State between 2018 and 2023. Sequence type (ST) 1 and ST2 were predominant, comprising 52.7% and 45.5% of the total population, respectively. We identified seven genomic mutations, eight plasmid replicon types, and fourteen acquired genes that are associated with antimicrobial resistance (AMR), with 82.7% of the population possessing at least one of these AMR determinants. The most common resistance determinant (detected in 73 genomes) was the S83F amino acid substitution in DNA gyrase subunit A, which confers both quinolone and triclosan resistance. Nine resistance genes (aph(3")-Ib, aph(6)-Ib, blaCTX-M-15, blaTEM-1, catA1, dfrA7, qnrS1, sul1, and sul2) were found more frequently in ST1 than in ST2 and exhibited significant co-occurrence with each other. Furthermore, the two STs differed in time to their last common ancestor, genotype clusters, effective population size over time, and changes in fitness over time, indicating sequence type-specific trends in population dynamics. Understanding the circulation of resistance determinants and microevolutionary changes in STy is essential for implementing meaningful public health strategies to minimize the broader dispersal and persistence of high-risk multidrug resistant strains of this important pathogen.

REGULATED CONDENSATE FORMATION GOVERNS PYRIN INFLAMMASOME ASSEMBLY AND SIGNALING

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Pyrin is a key immune sensor in the inflammasome pathway that is historically associated with the most common autoinflammatory disease, Familial Mediterranean Fever (FMF). Its prevalence is linked to the bubonic plague epidemics caused by *Yersinia pestis*: pyrin gain-of-function mutations were selected as they conferred enhanced immune defense against *Y. pestis*, inadvertently leading to FMF development. Pyrin functions by detecting pathogen-induced disturbances in the actin cytoskeleton. Under normal conditions, pyrin is phosphorylated on its intrinsically disordered region (IDR) by protein kinase N (PKN) and sequestered in an inactive state by phospho-binding proteins 14-3-3s. Pathogens target RhoA GTPase to suppress actin-dependent immune responses. This is detected by pyrin, as inactive RhoA causes PKN inactivity and thus 14-3-3 dissociation, triggering inflammasome activation. However, the biochemical mechanisms underlying pyrin function and inhibition remain unknown. We discovered that pyrin's IDR promotes the formation of pyrin filaments. Our cryo-EM analysis of the filament revealed its role as a conserved functional scaffold, formed by pyrin PYD domain, for inflammasome assembly. We found that pyrin forms condensates *in vitro* and in cells. This process was mediated by specific charge patterns in the IDR and by inter-domain cooperativity, and was enhanced by FMF mutations. Notably, pyrin condensate drives the recruitment of the inflammasome adaptor ASC and effector pro-caspase-1 *in vitro*, indicating functional inflammasome assembly. Disrupting pyrin condensate formation inhibited pyrin signaling in THP-1 macrophages. Furthermore, 14-3-3 binding to pyrin IDR suppresses condensate formation, filament assembly, and ASC recruitment, revealing a mechanistic basis for the established 14-3-3-mediated pyrin inhibition. Altogether, we uncover the molecular mechanisms underlying pyrin inflammasome regulation, showing that controlled pyrin condensate formation governs pyrin assembly and signaling. Our findings provide mechanistic insights into innate immune signaling and helps advance the broader understanding of condensate function in cellular pathways.

TLR PRIMING LICENSES NAIP INFLAMMASOME ACTIVATION BY IMMUNOEVASIVE LIGANDS

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NLR family, apoptosis inhibitory proteins (NAIPs) detect bacterial flagellin and structurally related components of bacterial type III secretion systems (T3SS), and recruit NLR family CARD domain containing protein 4 (NLRC4) and caspase-1 into an inflammasome complex that induces pyroptosis. NAIP/NLRC4 inflammasome assembly is initiated by the binding of a single NAIP to its cognate ligand, but a subset of bacterial flagellins or T3SS structural proteins are thought to evade NAIP/NLRC4 inflammasome sensing by not binding to their cognate NAIPs. Unlike other inflammasome components such as NLRP3, AIM2, or some NAIPs, NLRC4 is constitutively present in resting macrophages and not known to be induced by inflammatory signals. Here, we demonstrate that Toll-like receptor (TLR)-dependent p38 mitogen-activated protein kinase signaling up-regulates NLRC4 transcription and protein expression in murine macrophages, which licenses NAIP detection of evasive ligands. In contrast, TLR priming in human macrophages did not up-regulate NLRC4 expression, and human macrophages remained unable to detect NAIP-evasive ligands even following priming. Critically, ectopic expression of either murine or human NLRC4 was sufficient to induce pyroptosis in response to immunoevasive NAIP ligands, indicating that increased levels of NLRC4 enable the NAIP/NLRC4 inflammasome to detect these normally evasive ligands. Altogether, our data reveal that TLR priming tunes the threshold for the murine NAIP/NLRC4 inflammasome to enable inflammasome responses against immunoevasive or suboptimal NAIP ligands. These findings provide insight into species-specific TLR regulation of NAIP/NLRC4 inflammasome activation.

LISTERIA MONOCYTOGENES RESILIENCE DURING INFECTION IS MEDIATED *VIA* STRESS-DEPENDENT ACTIVATION OF THE VIRULENCE PROGRAM.

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Listeria monocytogenes is a Gram-positive, facultative intracellular pathogen that expresses PrfA, the master virulence regulator essential for bacterial survival during each stage of its intracellular lifecycle. Transcription of *prfA* is initiated from several promoters one of which is regulated by Sigma B, an alternative Sigma factor that regulates the general stress response. The *L. monocytogenes* Sigma B regulon contains over 300 genes known to respond to different stressors. However, the role of Sigma B in the regulation of *prfA* during the infection remains unclear. To define pathways that lead to Sigma B-dependent *prfA* activation, we constructed a transposon library in a strain that has only a Sigma B-dependent promoter directly upstream of *prfA* and screened for mutants with virulence defects in a L2 fibroblast infection model. Our screen identified a number of mutants in a large multicomponent bacterial stress-sensing machinery known as the stressosome. Mutations in structural and regulatory components of the stressosome resulted in heterogeneity within bacterial populations with some bacteria behaving like wild type while other members of bacterial population exhibited defects in intracellular growth and cell-to-cell spread. The stressosome mutants were also impaired in vacuolar escape and growth in macrophages and were highly attenuated in mice. Taken together, we concluded that the stressosome plays a role in controlling bacterial homogeneity and contributes to robust virulence activation during infection. Future work will focus on understanding the host stress signals necessary for stressosome-dependent *prfA* activation during *L. monocytogenes* pathogenesis.

A PROSPECTIVE GENOME-WIDE ASSOCIATION STUDY OF COLONIZING VS INFECTING S. AUREUS IN MAJOR SPINE SURGERY

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Surgical site infection (SSI) is a devastating complication of surgery occurring in 1/30 procedures. Most arise endogenously from S. aureus strains colonizing 1 in 3 patients prior to surgery. Genetic variation among colonizing S. aureus strains (e.g. the mecA methicillin-resistance gene) is known to influence propensity for infection. However, prior studies have been limited by confounding from bacterial population structure, sample size, retrospective designs, or lack of adjustment for host susceptibility or procedural factors known to influence SSI risk.

Methods: We prospectively collected preoperative nasal and skin screening samples from patients undergoing spine surgery at Harborview Medical Center from 2019-2024. In addition to preoperatively colonizing strains, all S. aureus pathogens postoperatively cultured from surgical wounds in cases of SSI were retrieved from the clinical microbiology lab. Isolates were subject to whole-genome sequencing and partitioned as follows:
(a) Discovery set: case and control isolates, not requiring propensity matching and including additional historical case samples from the same clinical population prior to the start of prospective screening
(b) Held-out validation set: each case (colonized → surgery → SSI) propensity matched to one or more controls (colonized → surgery → no SSI) using patient- (diabetes, obesity, immunosuppression, etc) and procedural (duration, implant use, surgical approach, etc) factors
We conducted genome-wide association studies using pyseer to adjust for bacterial population structure and examine multiple forms of genetic variation (gene presence-absence, non-synonymous variation, and kmer-based comparisons). Sequences were also compared to assess molecular epidemiology across patients and test for convergent evolution in the progression from colonization to infection within individuals.

Results: 193 S. aureus SSI (case) and 411 colonizing (control) isolates were successfully sequenced from 1,091 patients. Preoperative colonization (28% of patients) was confirmed as a risk factor for SSI with most arising endogenously and no evidence of common-source infection across patients. After adjustment for clinical risk factors, phage-derived leukocidins conferred the greatest independent risk for SSI, followed by MSCRAM polymorphisms and membership of specific clades.

Conclusions: Phage-encoded immune evasion genes dominate the landscape of bacterial genetic risk factors for S. aureus SSI, conferring significant risk beyond host- and procedural factors.

TARGETING AND REGULATION OF EPSTEIN-BARR VIRUS ORIGIN OF DNA REPLICATION BY HSA-MIR-155

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MicroRNAs (miRNAs) are small non-coding RNA molecules found in most eukaryotic cells, known to regulate gene expression post-transcriptionally through mRNA degradation and translational repression. Several reports suggested that viral infection could suppress host cellular function via miRNA approaches. Here, this study presents a new function of miRNAs in regulating DNA replication of both virus and host cell. A set of cellular miRNAs capable of repressing the origin of DNA replication (oriP) of the Epstein-Barr virus (EBV) has been identified. Among them, miR-155 directly binds to the dyad symmetry (DS) sequence of oriP, competing with EBNA1, the essential viral protein for latent phase replication, thereby repressing DNA replication. When the binding of miR-155 to the DS sequence is disrupted by mutation, EBNA1 can bind to the DS sequence, and replication resumes. These findings reveal a new mechanism by which miRNAs repress DNA replication through direct interaction with the replication origin sequence. Our findings also shed light to a novel pathogenesis of latent viral infection.

ORAL INTERFERON-GAMMOPATHY ALTERS *CANDIDA ALBICANS* PATHOGENESIS

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Candida albicans is a pathobiont— it is adept at colonizing mucosal sites and causing mucocutaneous and systemic infection. While mucocutaneous candidiasis is usually mild and treatable, chronic mucocutaneous candidiasis (CMC) occurs when the immune system is unable to control the yeast. One group of patients which is susceptible to CMC is patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). APECED is a genetic disease caused by deficiency of the transcription factor autoimmune regulator (*AIRE*). In the absence of *AIRE*, multiorgan autoimmunity develops, involving excessive interferon-γ (IFN-γ), termed interferon-gammopathy. This excessive IFN-γ causes epithelial cell death and barrier disruption in the oral mucosa, and susceptibility to candidiasis.

We are studying how *C. albicans* can take advantage of oral interferon-gammopathy to cause disease in *Aire* KO mice, a model for APECED. Intriguingly, we have found that key genes necessary for *C. albicans* pathogenesis in other contexts are dispensable for high levels of colonization in the *Aire* KO mouth, including *EFG1*, *BRG1*, *HGC1* and *ECE1*. Further, mutants *bcr1*ΔΔ, *tec1*ΔΔ, and *rob1*ΔΔ, which have been shown to display lower fungal burdens in the cortisone model of oral candidiasis, are able to colonize to high levels in the *Aire* KO mouth. Thus, mechanisms of *C. albicans* survival in the environment of interferon-gammopathy are different to those in immunosuppression. However, when *Aire* KO mice are infected with *efg1*ΔΔ, *hgc1*ΔΔ, or *ece1*ΔΔ, they lose less weight, show less pathology, and exhibit less type 17 inflammation, consistent with commensal oral colonization. Thus, these virulence mechanisms are indeed necessary for pathogenesis during oral interferon-gammopathy. We are also studying the transcriptional response of two different *C. albicans* strains to oral interferon-gammopathy, and preliminary analysis revealed that they have different responses to the same environment. We are further using metabolomics to define the soluble mediators present in saliva from *Aire* KO mice that may trigger these transcriptional responses in *C. albicans* during infection. This work extends our understanding of *C. albicans* pathogenesis to an autoinflammatory environment.

This research was supported by the Intramural Research Program of NIAID and the Postdoctoral Research Associate Training program of NIGMS.

TRIM14 IS A MASTER REGULATOR OF STAT3 ACTIVITY DURING THE MACROPHAGE INNATE IMMUNE RESPONSES TO *MYCOBACTERIUM TUBERCULOSIS*

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Despite our extensive knowledge of cell death pathways, how these pathways are shaped by host determinants during *Mycobacterium tuberculosis* (Mtb) infection remains unclear. Cell death is a critical determinant of Mtb infection outcomes, where Mtb has evolved ways to block apoptosis and push cells towards necrosis that allows for enhanced Mtb dissemination and pathology. Tripartite motif (TRIM) proteins are central regulators of innate immunity and modulate cell death pathways through interactions with components of these signaling networks. Our previous work has shown that TRIM14 interacts with TBK1 and modulates its kinase activity on the transcription factor STAT3 to promote phosphorylation and activation of STAT3. While STAT3 activity has been shown to regulate proliferation and cell death, it also has non-conventional roles in regulating mitochondria homeostasis by localizing to the mitochondria. We hypothesized that TRIM14 may regulate STAT3 activity in a TBK1-dependent manner that dictates cell death outcomes during Mtb infection. To better understand regulation of cell death modalities, we used *Trim14*$^{-/-}$ *ex vivo* bone marrow-derived macrophages (BMDMs) to investigate whether TRIM14 regulates programmed cell death (apoptosis) or necrosis. We found that *Trim14*$^{-/-}$ macrophages significantly increase apoptosis in response to Bcl2 inhibitors and Mtb infection in a STAT3 dependent manner, whereas necrotic cell death outcomes remained unchanged. Remarkably, overexpression of TRIM14 dramatically reduces apoptotic cell death during Mtb infection. We hypothesize that diminished STAT3 activity at the level of the mitochondria in these cells may contribute to enhanced apoptosis. Given the protective role of apoptosis during Mtb infection, we used *in vivo* aerosol infection models to investigate how TRIM14 influences disease outcomes. We found that *Trim14*$^{-/-}$ mice survived longer during Mtb infection and associate with protective responses which include higher cross presentation by APCs to CD8+ T cells and higher levels of memory associated T cell responses. Here, we have identified TRIM14 as a key regulator of host cell death during Mtb infection, where loss of TRIM14 confers protection in mice by increasing macrophage sensitivity to apoptotic cell death. This work will further our understanding of how TRIM14 modulates STAT3 activity to regulate cell death outcomes, which may lead to possible therapeutic approaches to improve tuberculosis patient outcomes.

LOSS OF FUNCTION OF METABOLIC TRAITS IN TYPHOIDAL *SALMONELLA* WITHOUT APPARENT GENOME DEGRADATION

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Salmonella enterica serovar Typhi and Paratyphi A are the causing agents of typhoid and paratyphoid fever in humans, which are systemic deadly conditions. The two serovars have adapted to infect exclusively the human host, causing long-lasting persistent infections with indistinguishable clinical manifestations. A distinct feature of these serovars, in contrast with *Salmonella* enterica serovars that infect a broad range of hosts, is the presence of a relatively high number of degraded coding sequences coding for metabolic pathways, most likely a consequence of their adaptation to a single host. As a result of convergent evolution, these serovars shared many of the degraded coding sequences although often affecting different genes in the same metabolic pathway. However, there are several coding sequences that appear intact in one serovar while clearly degraded in the other, suggesting differences in their metabolic capabilities. In this work, we examined the functionality of metabolic pathways that appear intact in *S.* Typhi but that show clear signs of degradation in *S.* Paratyphi A. We found that, in all cases, the presence of single amino acid substitutions in *S.* Typhi metabolic enzymes, transporters, or transcription regulators resulted in the inactivation of these metabolic pathways. We demonstrate that the inability of *S.* Typhi to metabolize glucose-6-phosphate or 3-phosphoglyceric acid is due to the silencing of the expression of the genes encoding the transporters for these compounds due to point mutations in the transcriptional regulatory proteins. Alternatively, its inability to utilize glucarate or galactarate is due to the presence of point mutations in the transporter and enzymes necessary for the metabolism of these sugars. Our findings provide additional support for the concept of adaptive convergent evolution of these two human-adapted *S.* enterica serovars and highlight a limitation of bioinformatic approaches to predict metabolic capabilities.

MYCOBACTERIUM TUBERCULOSIS SECRETED EFFECTOR PROTEIN PE5 MODULATES MACROPHAGE ENDOSOMAL RECYCLING VIA CRL2 UBIQUITIN MACHINERY

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is the leading infectious killer and infects one quarter of the global population. During infection, Mtb uses secreted effector proteins to interfere with, modulate, and protect from potent antibacterial responses, allowing it to multiply and disseminate. One large family of mycobacterial effector proteins are the PE/PPE proteins, which are encoded by an impressive 10% of the Mtb genome. Because this gene family is so large specifically in pathogenic mycobacteria and because some PE/PPEs have demonstrated roles in immune evasion, they are believed to be critical for Mtb virulence and pathogenesis. However, the cellular functions of each of the 168 PE/PPE proteins have yet to be comprehensively characterized, at least in part because of their high GC content and large regions of extremely repetitive sequences. We discovered that one member of this family, PE5, promotes bacterial replication by hijacking the host Cullin-2 RING E3 ligase complex (CRL2). CRL2 normally maintains protein homeostasis by targeting misfolded or mistranslated proteins for proteasomal degradation. PE5 interacts with the CRL2 complex through its substrate receptor KLHDC2, which recognizes proteins ending in a Gly-Gly motif. PE5, which contains a C-terminal Gly-Gly, binds within the substrate-binding pocket of KLHDC2. Although this interaction does not result in PE5's ubiquitination, likely due to the absence of lysine residues in PE5, it nevertheless leads to PE5's proteasomal degradation. This raises the intriguing possibility that PE5 functions as a molecular adaptor, co-opting the CRL2 complex to redirect ubiquitination toward non-canonical substrates. By bridging CRL2 to new target proteins, PE5 may facilitate their degradation, representing a novel strategy for innate immune evasion. Intriguingly, we identified specific interactions between PE5 and the endosomal trafficking proteins VAMP8 and STX7, which mediate phagosome–lysosome fusion. Consistent with this, macrophages expressing PE5 show increased replication of *Salmonella*, indicating PE5-induced disruption of phagosome–lysosome fusion that promotes bacterial survival. Moreover, we observed mislocalization of the plasma membrane iron exporter FPN1, suggesting global defects in endosomal trafficking and recycling, likely driven by multiple interactions between PE5 and components of the endosomal machinery. We are now exploring how mechanistically PE5 hijacks the CRL2 complex to block endosomal trafficking and recycling. These mechanistic insights into PE/PPE-mediated innate immune evasion will identify host proteins that could be exploited for host-directed therapies against Mtb infection.

DAMAGE REPAIR IN *STAPHYLOCOCCUS AUREUS* FLUOROQUINOLONE PERSISTERS

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Bacterial persisters are phenotypic variants within genetically identical populations that survive antibiotic treatment. Persisters may contribute to recurrence and resistance development in infections caused by many bacteria such as *Staphylococcus aureus*. This study investigates how *S. aureus* persisters survive treatment with fluoroquinolones (FQs) including delafloxacin, a topoisomerase inhibitor recently approved for *S. aureus* treatment. We found that *S. aureus* persisters from stationary-phase cultures surviving FQ treatment require DNA double-strand break (DSB) repair for survival. Our data suggest that many *S. aureus* cells remain viable during FQ treatment but need recA-mediated DNA repair during recovery after drug removal. We also observed that post-treatment nutrient availability influences persistence. Starving delafloxacin- or ciprofloxacin-treated populations for 4 hours following treatment before nutritive recovery increased persistence by ~10-fold. Further, our data suggests that starvation after FQ treatment delays the resumption of nucleic acid synthesis. Indeed, inhibiting RNA or DNA synthesis in the presence of nutrients following treatment increased ciprofloxacin persistence to levels observed in starved populations. We propose that post-treatment starvation promotes *S. aureus* survival by allowing time for FQs to dissociate from topoisomerases before nucleic acid synthesis resumes, preventing catastrophic fork collapses. These findings highlight the importance of the timing of events during recovery after antibiotic treatment in modulating persistence phenotypes. They also suggest that targeting molecular events during this recovery period may lead to the development of more effective anti-*S. aureus* treatment strategies.

NEONATAL COLONIZATION BY *CLOSTRIDIOIDES DIFFICILE* FACILITATES SYSTEMIC SECONDARY INFECTION

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Clostridioides difficile is the most commonly reported nosocomial pathogen in adults in the United States and an urgent public health threat worldwide. Surprisingly, infants carry *C. difficile* at an incredibly high rate, yet are clinically asymptomatic. At the Children's Hospital of Philadelphia, we find that opportunistic pathogens, including *Streptococcus agalactiae* (Group B *Streptococcus*; GBS) and *Escherichia coli*, are often found in the stool of both healthy and high-risk infants that have been colonized by *C. difficile*. GBS and *E. coli* are leading causes of neonatal bacteremia and meningitis, however, the mechanisms facilitating translocation of these gut-dwelling pathogens into the blood remain poorly understood. Moreover, the effect of *C. difficile* on disease caused by other early life pathogens has also not been explored. Here, we report the development of a murine model for neonatal *C. difficile* intestinal colonization and secondary infection and demonstrate significant consequences of early life *C. difficile* colonization. We postulated that *C. difficile*-mediated intestinal barrier disruption would facilitate translocation of other pathogens that co-colonize the neonatal intestinal tract. In support of this, we observe enhanced systemic dissemination of GBS in *C. difficile*-carrying neonatal mice to extraintestinal organs such as the brain. Furthermore, *C. difficile* toxemia results in disruption of the blood-brain barrier. Using biopsy-derived human infant colonic epithelial cell line (HICECs) and a human cerebral microvascular endothelial cell (HCMEC) model of the blood-brain barrier, we further demonstrate that *C. difficile* toxins cause cell death and disruption of barrier function in both systems. Additionally, toxin treatment results in a molecular priming of these cells which enhances adherence of multiple GBS and *E. coli* strains. Collectively, these data demonstrate that neonatal colonization with *C. difficile* is not truly asymptomatic and represents a potential predisposing factor for systemic infection by high-risk neonatal pathogens.

A MANGANESE-SPARING RESPONSE BALANCES COMPETING CELLULAR DEMANDS TO ENABLE *STAPHYLOCOCCUS AUREUS* INFECTION

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Responding to stress is critical to the survival of life, especially for microbes that have a limited ability to manipulate their environment. During infection, *Staphylococcus aureus* and other invaders must overcome both the host-imposed absence of manganese and the oxidative burst of immune cells, which increases the need for this essential metal. The current investigations revealed that a small RNA, RsaC, integrates the staphylococcal responses to manganese starvation and oxidative stress. Upon manganese limitation, RsaC activates a manganese-sparing response, which decreases the cellular demand for manganese, enabling growth in manganese-restricted environments. However, the benefit of this response is environment-dependent as RsaC suppresses the expression of the manganese-dependent superoxide dismutase SodA, sensitizing *S. aureus* to oxidative stress. Despite this suppression, RsaC is necessary for *S. aureus* to cause infection, with its importance dependent on the efficacy of the host's manganese withholding response. These results reveal a previously unappreciated manganese-sparing response that is important for bacterial virulence, and the imperative role of sRNAs in balancing bacterial adaptation to stressors that place conflicting demands on cellular physiology.

TLR2 PROMOTES MACROPHAGE-MEDIATED CONTROL OF *ASPERGILLUS FUMIGATUS* IN LARVAL ZEBRAFISH

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Aspergillus fumigatus is a ubiquitous fungus that is non-threatening to healthy individuals; however, *A. fumigatus* infection can become fatal in immunocompromised individuals. Early recognition of infection by innate immune cells, including macrophages, is essential for pathogen killing. Toll-like receptor 2 (Tlr2) is an extracellular pattern recognition receptor (PRR) that can co-localize with *A. fumigatus* spores in macrophages in cell culture, but the importance of Tlr2 for spore clearance and host survival *in vivo* is not yet understood. To investigate this question, we use a larval zebrafish model host which is ideal for replicating human infection due to their genetic similarity to humans with about 70% of human genes having at least one orthologue in zebrafish. Larval zebrafish also have the capability for high resolution live-organism imaging and simple genetic manipulation with tools such as CRISPR/Cas9 and Tol2 transgenesis. Using this model, we find that Tlr2 promotes host survival against *A. fumigatus* and promotes spore clearance. Additionally, we found that targeting *tlr2* with gRNA significantly decreases the number of macrophages recruited to the site of infection at 1- and 2-days post infection but does not impact neutrophil recruitment. These data suggest that Tlr2 specifically promotes spore clearance through macrophage function. Our data highlights a significant role of Tlr2 in immune function during *A. fumigatus* infection. Currently, we are investigating how Tlr2 impacts phagocytosis and spore trafficking in macrophages. Findings from these experiments will further elucidate how Tlr2 functions during *A. fumigatus* infection.

THE CSPC:CSPA HETERODIMER TRANSDUCES GERMINANT AND CO-GERMINANT SIGNALS DURING *C. DIFFICILE* SPORE GERMINATION.

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Clostridioides difficile is a gastrointestinal pathogen that is the leading cause of nosocomial infections in the United States and many developed countries. *C. difficile* infection begins when its metabolically dormant spores encounter gut-specific small molecules that trigger the process of germination. In *C. difficile*, this process occurs via a unique mechanism where *C. difficile* lacks the transmembrane receptors conserved among almost all spore-forming bacteria, and requires two signals to initiate germination: a bile acid germinant and an amino acid or divalent cation co-germinant signal. While two soluble pseudoproteases, CspC and CspA, were initially implicated in sensing germinant and co-germinant signals, respectively, we previously showed that CspC modulates the sensitivity of *C. difficile* spores to both signals via a flexible loop. Here, we show that CspC forms a stable complex with CspA and that this complex, rather than the individual proteins, is responsible for integrating germinant and co-germinant signals. Guided by our crystal structure of the CspC:CspA heterodimer, our structure-function analyses revealed that CspC's flexible loop interacts with CspA and allowed for the identification of interactions at the CspC:CspA interface that regulate the sensitivity of *C. difficile* spores to germinant signals. These analyses revealed for the first time that CspA regulates *C. difficile's* response to bile acid germinant. While we also showed that CspA can form a homodimer and solved its crystal structure, we found that disrupting CspA homodimerization minimally impacts *C. difficile* spore germination. Collectively, our analyses establish the CspC:CspA heterodimer as a critical signaling node for sensing germinant and co-germinant signals and provide novel structural insight into the unique germination mechanism of this clinically significant pathogen.

DEFINING HUMAN RESPIRATORY SYNCYTIAL VIRUS MEDIATED HOST IMMUNE MODULATION WITH MUTANT VIRUSES

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Human respiratory syncytial virus (hRSV) is a leading cause of lower respiratory tract infections in infants, young children and older adults living with comorbidities. Globally, each year hRSV is responsible for 33 million cases of infections with 3.6 million hospitalizations and more than 100,00 deaths in children younger than 5 years. Early life hRSV infections appears to gain epigenetic control of innate immunity and may contribute to long-term respiratory sequelae. In recent years, significant efforts have led to the development of prophylactic and therapeutic treatment options, however continued efforts towards elucidating host immune responses during infections are necessary to prevent long-term lung impairment in infected individuals.

hRSV is a non-segmented, negative sense RNA virus that encodes two multifunctional nonstructural proteins – NS1 and NS2. Previous research has identified that these hRSV encoded proteins are involved in viral-mediated host immune antagonism. Previous work from our lab have found that NS1 translocates to the nucleus where it interacts with host chromatin thus modulating host gene expression. Furthermore, work from our lab identified key residues within hRSV NS1 involved in immune antagonism. Reverse genetics is a powerful tool that has been employed to manipulate viral genome and study the effect of mutations in the genome on growth characteristics of viruses. Here, we have employed circular polymerase chain reaction (CPER) to develop a panel of hRSV mutants that limit their host interactions. Through these mutant viruses, we are investigating the role of hRSV NS1 in remodeling airway epithelium and in epigenetic control of immunity. We have assessed the functional consequences of these mutations through RNA sequencing to determine the role of specific host-viral interactions during hRSV infection.

INVESTIGATING MECHANISMS OF ANTIBIOTIC TOLERANCE IN *STAPHYLOCOCCUS AUREUS*

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Staphylococcus aureus is a leading cause of osteomyelitis (OM), a highly morbid infection of bone. Despite the administration of appropriate antibiotics, treatment failure occurs in up to ~20% of the cases. Recently appreciated as a mechanism of treatment failure, antibiotic tolerance refers to the ability of bacteria to survive lethal concentrations of antibiotics without an increased minimum inhibitory concentration (MIC). To model antibiotic tolerance, our lab has developed a robust murine OM model that recapitulates the extreme treatment recalcitrance observed clinically in patients with bone infection. To identify bacterial genes contributing to tolerance in OM, a transposon sequencing (Tnseq) experiment was performed, revealing that inactivation of the transcriptional repressor *purR* enhances bacterial survival during antibiotic treatment. Based on the established role of PurR in regulation of purine biosynthesis and phosphotransferase system (PTS) activity, we hypothesized that inactivation of *purR* alters bacterial metabolism in ways that render *S. aureus* more tolerant to antibiotics. To confirm the Tnseq findings, stationary cultures of LAC and LAC *purR::Tn* were challenged with vancomycin at 50x MIC. Results indicate that LAC *purR::Tn* exhibits a significant increase in survival compared to its isogenic wild-type strain 72h post treatment. MIC values for both the WT and mutant strains remained unchanged, suggesting that resistance was not responsible for the enhanced survival of the *purR* mutant. This phenotype was reversed by complementation of the native *purR* gene and promoter into the chromosome. Additionally, upon screening ~300 clinical isolates, one isolate from a patient with osteomyelitis was found to harbor a missense mutation in *purR*. This isolate displayed modestly heightened survival compared to LAC. Future research will test the hypothesis of whether increased purine biosynthesis or PTS activity underlies enhanced tolerance. Understanding the changes in bacterial metabolism that support antibiotic tolerance may assist in developing strategies to combat persistent *S. aureus* infections, such as osteomyelitis.

HOST RESPONSES TO SPECIFIC VIRULENCE DETERMINANTS HELP DEFINE MECHANISMS OF *RICKETTSIA RICKETTSII* VIRULENCE.

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Rickettsia rickettsii is the causative agent of Rocky Mountain Spotted Fever, a potentially fatal tickborne illness. Previous work highlighted the utility of primary human dermal microvascular endothelial cells (HDMECs) as a restrictive cell culture model that differentiates avirulent and virulent *R. rickettsii* strains. HDMECs support robust growth of the virulent *R. rickettsii* Sheila Smith (SS) but not the avirulent Iowa strain. Interferon-β (IFN-β) responses are highly upregulated during Iowa infections of HDMECs and correlate with inhibition of replication and host cell lysis. In contrast, SS infections lead to delayed synthesis and reduced amounts of IFN-β. We conducted a temporal transcriptional analysis of avirulent and virulent *R. rickettsii* strains as well as recombinant strains expressing unique rickettsial virulent determinants. Major pathways differentially induced between SS and Iowa infections included type I interferon signaling and the unfolded protein response (UPR). The increased IFN-β secretion by Iowa infection enhances interferon regulatory factor 9 (IRF9) nuclear translocation at earlier timepoints of infection than SS infected cells. Furthermore, siRNA-mediated knockdown of IRF9 limits interferon-stimulated gene expression and enhances Iowa replication. IFN-β synthesis is inhibited by expression of the rickettsial autotransporter peptidase lipoprotein (RapL) which cleaves surface-exposed passenger domains of autotransporters and is not expressed by Iowa. RapL is not secreted thus, it is proposed that one of the four rickettsial autotransporter passenger domains may be responsible for the downregulation of IFN-β. RapL expression in the Iowa strain limits *IFNB1* expression and delays phosphorylation of signal transducer and activator of transcription (STAT) proteins 1-2. The rickettsial ankyrin repeat protein 2 (RARP2) catalyzes disruption of the trans-Golgi network and inhibits export of secreted proteins, including IFN-β. RARP2 is truncated and inactive in the Iowa strain while the full-length active form is expressed and secreted by SS. RARP2 expression by the Iowa strain decreases IFN-β secretion, activates the UPR, enhances nuclear translocation of the pro-apoptotic transcription factor DDIT3, and may ultimately lead to the late-stage apoptosis observed in SS infected cells. Independently, and potentially synergistically, these virulence determinants influence interactions within the eukaryotic host cell and provide important clues to mechanisms of rickettsial pathogenesis.

ENDOCANNABINOID SIGNALING AT CANNABINOID RECEPTOR 1 ENHANCES ILEAL PROINFLAMMATORY TONE AND PROMOTES MICROBIAL DYSBIOSIS

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Crohn's disease (CD) is characterized by chronic intestinal inflammation and microbial dysbiosis with disease often manifesting in the ileum. The endocannabinoid system (ECS), comprised of lipid hormones (endocannabinoids) and cannabinoid receptors (e.g. CB1), modulates host metabolism and immunity. Clinical multi-omics studies reveal positive correlations between gut endocannabinoid levels and microbial dysbiosis in active CD. To establish whether ECS signaling alters the ileal microbiome, non-inflamed wild type (WT) mice were treated with an inhibitor that blocks Magl-mediated degradation of endocannabinoid 2-AG, promoting its accumulation to stimulate ECS signaling. Magl inhibition induced significant microbiome changes resembling dysbiosis often observed in CD, including increased *Enterococcus*, *Escherichia coli*, and *Turicibacter* and decreased *Lachnospiraceae*. Magl inhibitor administered with a CB1 antagonist, or in CB1-deficient mice, prevented microbial dysbiosis. In CD-susceptible, pre-colitic *Il10*-deficient mice, Magl inhibition stimulated *E. coli* pathobiont proliferation and mucosal colonization in a CB1-dependent manner. To determine how CB1 activation alters the microbiome, we performed RNA-seq on ileal lamina propria collected from WT mice prior to observed microbial changes. GSEA pathway analyses revealed Magl inhibition upregulated several immune pathways including INF-γ, TNF-NFκB, and IL6-JAK-STAT3 in a CB1-dependent manner. We therefore hypothesized that CB1 signaling enhances ileal proinflammatory tone, resulting in luminal influx of inflammation-associated nutrients such as oxygen and nitrate that favor growth of respiring bacteria such as *E. coli*. Supporting this idea, ileal expression of inducible nitric oxide synthase *Nos2*, which can increase luminal nitrate concentrations, was significantly upregulated. Enterobacteriaceae-free WT mice were treated with Magl inhibitor following engraftment of commensal *E. coli* WT or respiration mutants. Proliferation of both nitrate (Δ*narGnarZ*) and aerobic (Δ*cydAB*) respiration mutants were significantly decreased following Magl inhibition. Similarly, a *Nos2* inhibitor significantly attenuated ileal *E. coli* growth stimulated by Magl inhibition. Together, our findings suggest that endocannabinoid activation of CB1 enhances ileal proinflammatory tone, thus altering the luminal nutrient environment and promoting ileal dysbiosis. More broadly, these findings suggest that enhanced ECS activity may contribute to CD pathogenesis, thus introducing a novel therapeutic entry point for managing disease.

BIOFILM DISPERSION IN *ENTEROCOCCUS FAECALIS* IS MEDIATED BY NUTRIENTS AND INTESTINAL METABOLITES

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Background: *Enterococcus faecalis* OG1RF is a Gram-positive intestinal commensal that can cause serious infections in immunocompromised individuals, including urinary tract infections, endocarditis, and wound infections. Its ability to form biofilms on medical devices contributes to persistence and antibiotic resistance, including the emergence of vancomycin-resistant enterococci (VRE) (1). Previous studies have examined biofilm assembly in *Enterococcus faecalis*, however, biofilm dispersion, the final step in the biofilm life cycle remains uncharacterized.
Methods: We optimized *in vitro* assays to OG1RF to assess biofilm dispersion. Candidate gene analysis was used to identify potential regulators.
Results: We confirmed that dispersion is triggered by a 10-fold step-change increase in nutrients. Dispersal events were confirmed with confocal microscopy. Typically, tolerance to antibiotics is decreased in dispersed cells and we confirmed this phenomenon for the clinically relevant antibiotics Ampicillin, Linezolid, Vancomycin and Daptomycin. Enterococci are common members of the gut microbiota of terrestrial coelomates and, in vertebrates, live in an environment with high levels of bile acids. We assayed the effects of a panel of bile acids on biofilm dispersion and found that physiological levels of the secondary bile acid lithocholic acid strongly inhibits biofilm dispersion. Finally, using a candidate gene approach, we identified two potential genetic regulators of biofilm dispersion. The peptidoglycan hydrolase *salA* and the manganese exporter *mntE*.
Conclusions: Taken together, our data suggest that biofilm dispersion in *E. faecalis* is modulated by nutrient availability and metabolites produced by the gut microbiota. For enterococci in the gut, the balance of biofilm formation, biofilm dispersion and antibiotics tolerance may be mediated by daily cycles of feeding and fasting.
1. Cetinkaya Y et al. Clin Microbiol Rev. 2000;13:686–707.

EXPLORING THE *STAPHYLOCOCCUS AUREUS* HOST-PATHOGEN INTERFACE USING DEGRADOMIC TECHNOLOGIES

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Much of the work on protease substrate identification has been via purified protein and immunoblotting, leading to a low-throughput, one-at-a-time approach. Herein, we developed a new N-terminomic methodology, TAGS-CR, to globally profile host targets of the *S. aureus* secreted proteases. Using two ex-vivo models - human neutrophils and human lung tissue – TAGS-CR captured ~670 targets for the V8 protease, revealing its role in manipulating host defense strategies in diverse host niches. In human neutrophils we found immune cell adhesion and migration to be targeted and observed dysregulation of oxygen-dependent and -independent leukocyte defenses. V8 may also facilitate bacterial dissemination via activation of leukocyte apoptosis. In human lung tissue, V8 influenced processes such as inflammation, nutritional immunity and tight junction formation. We also captured immunomodulatory effects of V8, as the complement system, immunoglobulins and neutrophil proteins were all targeted. We next applied TAGS-CR to in-vivo models of infection - murine sepsis and osteomyelitis - and observed conserved themes in host-pathogen interplay. During infection, we noted protease activity similarly promoted immune evasion via targeting of neutrophil defenses, including ROS production, degranulation, NET formation, migration and phagosome maturation. Inflammatory dysregulation was also observed via cleavage of complement proteins, immunoglobulins and SERPINS. Cytokine signaling and antigen presentation were also targeted. Tissue destruction was prominent, with proteolysis of muscle fiber constituents and various cytoskeletal proteins detected. In sum, degradomic approaches are a powerful tool for the uncovering of protease substrates, providing unique insight into the host-pathogen interface.

GUT DYSBIOSIS IN AXIAL SPONDYLOARTHRITIS AND IMMUNE-MODULATING THERAPEUTIC POTENTIAL OF MICROBIOME-DERIVED BUTYRATE

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Gut dysbiosis is increasingly recognized as an environmental factor influencing axial spondyloarthritis (axSpA) pathogenesis. This study aimed to explore the differences in gut microbiota composition between axSpA patients and healthy controls (HCs) and investigate associations among specific microbial species, their metabolites, and axSpA progression. Using 16S rRNA sequencing of fecal samples from 33 axSpA patients and 20 HCs, we characterized gut microbiome diversity. AxSpA patients exhibited reduced α-diversity, indicating lower microbial diversity compared to HCs. At the species level, axSpA patients had increased abundance of Bacteroides and Streptococcus, whereas the butyrate-producing bacterium Faecalibacterium prausnitzii was notably reduced compared to HCs. To elucidate the immunological role of F. prausnitzii, peripheral blood mononuclear cells (PBMCs) isolated from axSpA patients were treated with F. prausnitzii (0.1, 1, and 10 μg/mL) or butyrate (0.5 and 1 mM). Analysis revealed decreased differentiation of CD4+ IL-17A+ T cells and IL-17A production, along with increased differentiation of CD4+ CD25high Foxp3+ Tregs and IL-10 levels. Additionally, butyrate significantly inhibited osteoclastogenesis and CD8+ tissue-resident memory T (CD8+ TRM) cell activation. In curdlan-induced SpA mouse models, administration of F. prausnitzii or butyrate decreased CD4+ IL-17A+ T cell polarization and CD8+ TRM activation, while enhancing CD4+ CD25high Foxp3+ Tregs. Furthermore, butyrate treatment reduced arthritis severity and inflammation. Collectively, our findings suggest that decreased abundance of butyrate-producing microbes, particularly F. prausnitzii, contributes significantly to axSpA pathogenesis through modulation of T cell subsets, including IL-17A-producing T cells, Foxp3+ Tregs, and CD8+ TRM cells.

IN VIVO *IRGM1* DEFICIENCY IN CD11C^{+} CELLS DRIVES TYPE I INTERFERON-MEDIATED LUNG PATHOLOGY DURING TUBERCULOSIS.

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IRGM1 is a 47kDa interferon (IFN) inducible GTPase essential for controlling *Mycobacterium tuberculosis* (Mtb) infection in mice. It was originally shown that IRGM1 is recruited to Mtb containing phagosomes in infected macrophages where it was proposed to be involved in autophagy-mediated clearance of Mtb. However, we have previously discovered that autophagy degradation is not required in macrophages to control Mtb replication. In addition, it has since been reported that IRGM1 does not colocalize with phagosomes containing *Mycobacterium bovis* BCG in IFN-gamma treated cells, together suggesting that IRGM1 must have non-autophagic roles in tuberculosis disease. We dissected the contribution of IRGM1 to immune control of Mtb pathogenesis in vivo and found that germline deletion of *Irgm1* leads to higher levels of type I interferon signaling. The increased type I interferon signaling precludes T cell expansion during Mtb infection. The absence of Mtb-specific T cell expansion in *Irgm1*$^{-/-}$ mice results in uncontrolled Mtb infection in neutrophils and alveolar macrophages (AMs), which directly contributes to susceptibility to infection. The defect in T cell expansion due to the increased type I interferon was restored by deletion of another GTPase *Irgm3*, resulting in better Mtb control in neutrophils and AMs, as well as decreased type I interferon-stimulated gene expression. To determine what cell types require IRGM1 expression to control Mtb infection, we used an *Irgm1* reporter mouse that expresses DsRed as a transgene under control of the *Irgm1* promoter and found that lung macrophages and dendritic cells (DCs) are the primary cell types expressing IRGM1 in Mtb-infected mice. Indeed, deletion of *Irgm1* specifically in CD11c^{+} lung macrophages and DCs resulted in higher Mtb burdens in neutrophils and AMs in the lungs of infected mice as well as decreased mouse survival following Mtb infection. Loss of *Irgm1* in lung CD11c^{+} cells leads to delayed proliferation of Mtb-specific CD4^{+}T cells, resulting in delayed initiation of the adaptive immune response. We are continuing to dissect how IRGM1 in specific innate immune cell types regulates inflammation during pulmonary tuberculosis. Together, our studies reveal that IRGM1 is required in CD11c^{+} cells in the lung to mediate T cell-mediated control of Mtb infection in neutrophils and AMs, which is essential for the survival of Mtb-infected mice.

BIPA IS REQUIRED FOR INDUCTION OF VIRULENCE FACTOR EXPRESSION AND ENVIRONMENTAL ADAPTATION IN EHEC

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Enterohemorrhagic Escherichia coli 0157:H7 (EHEC) is a foodborne pathogen that poses a significant health risk due to its ability to generate attaching/effacing (A/E) lesions during intestinal colonization. These lesions are characterized by EHEC attachment to colonic epithelial cells, effacement of brush border microvilli, and the formation of actin-rich pedestals beneath the bacteria. We recently generated an EHEC mutant that lacks a ribosome-associated GTPase, BipA, to study virulence factor expression. BipA is a prokaryotic translation factor that binds to the ribosome and confers a growth advantage to bacteria by acting as a signaling intermediary between the ribosome and the environment. Strikingly, this EHEC deltaBipA mutant strain fails to form actin pedestals during infection of cultured human cells. These results raise the question of how changes in virulence factor expression negate the pedestal-forming ability of BipA-deficient bacteria. We addressed this by utilizing qRT-PCR, immunoblotting, and fluorescence microscopy to demonstrate that BipA influences the expression of virulence proteins encoded by the EHEC pathogenicity island, the Locus of Enterocyte Effacement (LEE), which contribute to actin pedestal-mediated colonization. In addition, proteomic profiling revealed broad changes in the abundance of proteins linked to the bacterial stress response. These findings indicate that BipA's influence extends beyond the local control of the LEE, to a global role in coordinating virulence and modulating bacterial cell homeostasis, positioning it as a key regulator of host-pathogen interactions. Identifying these activities by BipA may eventually provide a therapeutic opportunity for disrupting EHEC pathogenicity.

CITRAL AND GERANIOL: TWO NOVEL AND POTENT NON-CARCINOGENIC TERPENE ALTERNATIVES TO ALCOHOL-BASED MOUTHWASHES AGAINST CARIOGENIC ORAL BACTERIA

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Objective: Several recent studies have linked the high incidence of oral cancer among users with a history of prolonged use of alcohol-based mouthwashes, due to increased topical acetaldehyde, a carcinogen made from alcohol breakdown. Our research has previously shown that two volatile oils, *C. winterianus* (Lemon grass) and *C. flexuosus* (Citronella Java), exhibit significant antibacterial activity on four commonly occurring cariogenic oral bacteria. We have also demonstrated that these oils are mainly composed of four terpenes: Citronellal, Citronellol, Geraniol, and Citral. In this follow-up study, we wanted to determine which of these specific terpene constituents was responsible for the antibacterial activity of these two volatile oils.

Materials and Methods: In this study, we tested these terpenes on our selected panel of oral bacteria, namely *M. luteus*, *S. salivarius*, *S. mutans*, and *E. faecalis* in several qualitative assays such as Kirby-Bauer disc diffusion, Minimum Inhibitory Concentration (MIC) determination, Minimum Bactericidal Concentration (MBC) determination, and time-kill kinetics to evaluate their effectiveness as antibacterial agents.

Results: Citral and Geraniol emerged as the most potent broad spectrum antibacterial agents among all four terpene candidates. More specifically, Citral was most effective on both oral streptococcal strains followed by Geraniol, while Geraniol exhibited the highest antibacterial activity against *E. faecalis*. Both terpenes showed a similar pattern of killing as seen with oral streptococci when tested against *M. luteus*.

Conclusion: We hypothesize that these two terpenes may serve as safer, better alternatives to alcohol-based mouthwashes.

ISOLATES OF THE *CIONA ROBUSTA* GUT MICROBIOME REVEAL A SPECTRUM OF MECHANISTIC HOST-MICROBIOTA INTERACTIONS

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Ciona robusta is a marine invertebrate chordate (tunicate) that is considered an invasive species in many coastal areas of the world. Phylogenetically, it is the closest invertebrate relative to vertebrates. As a genetically tractable and high-throughput system, it has emerged as an important developmental model system, particularly in neurobiology, stem-cell biology, and gut microbiota-immune interactions. Despite its continuous filter-feeding lifestyle and a simpler innate immune system, *Ciona* maintains a distinct and compartmentalized gut microbiome. We have shown that, similar to mammalian systems, a significant portion of the main bacterial taxa in the gut are lysogenized, meaning they carry prophages integrated into their genomes. These prophages are part of the bacterial accessory genome, which can impact important traits that shape fitness outcomes. Our lab is leveraging the *Ciona* system to investigate the impact of prophages in host microbiomes. One such lysogen is *Shewanella fidelis* 3313; here, we demonstrate that one of its prophages has a profound effect on bacterial traits, including motility behaviors and biofilm formation, resulting in distinct colonization outcomes *in vivo*. The impact of this prophage also affects the recognition response of the *Ciona* immune system, including the expression of a secreted immunoglobulin-containing host effector molecule that can shape colonization dynamics. We find that this bacterial isolate also exists as two morphological variants, each with unique lytic phage susceptibilities and impacts on colonization behaviors. The *Ciona* system provides a high-throughput approach to studying colonization dynamics, revealing bacterial traits and phenotypes that are often undetected in traditional sequencing studies. Our research on the impacts of prophages among gut microbiota isolates may also help demonstrate how the accessory genome influences virulence phenotypes and host perception of them.

MICROGLIAL UPTAKE OF LISTERIA FROM NEURONS BOOST INFECTION

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Neurolisteriosis, caused by the invasion of the central nervous system (CNS) by the Gram-positive bacterial pathogen *Listeria monocytogenes* (Lm), affects both humans and farmed ruminants, posing a significant One Health concern. CNS macrophage populations, comprising resident microglia and infiltrating monocyte-derived macrophages (MDMs), adopt different roles in the defense against Lm. However, the interplay between these macrophage subsets and their interactions with infected neurons remain poorly understood, as does the impact of these interactions on the bacterial fate.

Our objective is to elucidate how these intercellular interactions shape the intracellular lifestyle of Lm in the CNS. In previous studies, we revealed that infected macrophages exhibit divergent transcriptional responses: microglia promote neutrophil recruitment while supporting a cytosolic, replication-permissive niche, whereas MDMs restrict bacteria to intravacuolar compartments. This restriction is associated with stress-related and SOS-response gene expression in Lm, indicating that the cellular context has a critical influence on bacterial adaptation.

To further dissect these dynamics, we have established co-culture systems combining microglia, MDMs, and a neuron-like cell line (FBBC), to simulate early stages of neuroinfection. We could observe that early addition of MDMs reduces bacterial burden by entrapping bacteria in vacuoles, but this fails to eliminate the infection, as Lm continues to replicate within the FBBC cytosol. In contrast, early microglial addition enhances Lm growth by actively acquiring bacteria from the FBBCs and promoting their intracellular replication. This effect is further confirmed using an actA-deletion mutant, which is incapable of forming actin tails, thus inhibiting bacterial spread via cell-to-cell transmission.

Live-cell imaging of the Lm actA-deletion mutant further confirms that microglia engage with Lm-infected FBBCs, while MDMs remain uninfected. This suggests that it is the microglia, rather than the MDMs, that play a critical role in facilitating early bacterial spread by directly acquiring the bacteria from infected neurons.

Overall, our results reveal that the diversity of the CNS macrophage population and its cellular interactions in the neuronal context sculpt Lm infection dynamics and bacterial adaptation strategies. The co-culture infection model presented here provides a valuable platform for gaining mechanistic insights into the impact of macrophage–neuron crosstalk on intracellular pathogen infections.

CONSUMPTION OF LOW-DIGESTIBLE CARBOHYDRATES INCREASES SUSCEPTIBILITY TO *SALMONELLA* TYPHIMURIUM INFECTION

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Low-digestible carbohydrates (LDCs), including dietary fibers and sugars that resist absorption, are generally considered beneficial for the gut microbiota. However, we show that ingestion of certain LDCs, particularly non-digestible sugars such as arabinose, lactulose, and polyols commonly used as sugar substitutes (e.g., erythritol, maltitol, and sorbitol), can compromise colonization resistance against the common foodborne pathogen *Salmonella enterica* serovar Typhimurium. Consumption of these sugars results in elevated fecal pathogen loads, systemic dissemination, and colitis in infected mice. Focusing on arabinose, we found that rapid fermentation of this sugar acidifies the intestinal environment and shifts the microbiota composition. Metabolomic profiling revealed altered short-chain fatty acid levels and elevated availability of amino acids, which serve as carbon and nitrogen sources for pathogen growth. Arabinose consumption also impacts virulence by broadening tissue tropism, enhancing systemic infection, and selecting for fully virulent, invasive Salmonella through modulation of microbiota–pathogen interactions. These findings reveal how diet can impact the gut microbiota in ways that enhance pathogen colonization and virulence, posing potential risks for enteric infections.

ROLE OF THE RCS PHOSPHORELAY IN *ENTEROBACTER CLOACAE* PATHOGENESIS

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ESKAPE pathogens are a group of microorganisms that have been deemed to be of utmost importance to study as they cause many antibiotic-resistant infections. Amongst these microorganisms is *Enterobacter cloacae*, a Gram-negative rod-shaped member of the order Enterobacterales. Normally a part of the human microbiota, it is increasingly becoming the culprit for many infections in patients who are hospitalized long-term and in immunosuppressed individuals. Despite contributing to the increasing number of infections that fail to respond to treatment, the pathogenesis of *E. cloacae* is largely unknown. In extensive preliminary work, we have employed a robust mouse model for chronic abscess infections by Enterobacterales to study *in vivo* antibiotic tolerance. Antibiotic tolerance is a phenotypic resistance characterized as the ability of bacteria to remain viable in the presence of antibiotics through damage repair functions. Damage repair, and therefore tolerance, is often mediated by stress responses, for example cell envelope stress response systems (CESR) in the case of cell wall-active antibiotics. Preliminary work revealed an interesting trade-off of a mutant in a prominent CESR. The Rcs phosphorelay caused lower *in vitro* tolerance to the last-resort beta-lactam antibiotic meropenem, but greater infectivity and a greater inflammatory phenotype in our mouse model. In the model organism *Escherichia coli*, Rcs regulates many clusters of genes, including colanic capsule production and motility, but its role in *E. cloacae* is unknown, both *in vivo* and *in vitro*. In this work, we begin to dissect the role of the Rcs system in *E. cloacae* pathogenesis and *in vivo* antibiotic tolerance. A known function of the Rcs regulon is motility, and infections in our mouse model with non-motile mutants indicate that it may be the primary driver of increased virulence. This work contributes to the gap of knowledge in *E. cloacae* pathogenesis and will allow for the exploration of new strategies to target Gram-negative bacteria through improved antimicrobial therapy.

LISTERIA MONOCYTOGENES VIRULENCE FACTOR INLB AS A KEY MODULATOR OF VERTICAL TRANSMISSION AND HUMAN TROPHOBLAST INFECTION.

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Listeria monocytogenes is associated with infections during pregnancy and maternal infection leads to fetal death as an outcome in approximately 20%-60% of reported cases. Much remains unknown regarding the mechanism(s) by which *L. monocytogenes* crosses the placental barrier to infect the developing fetus. We have previously identified a clinical isolate of *L. monocytogenes*, 07PF0776, that exhibits an enhanced rate of vertical transmission in mice that is dependent on the bacterial surface protein InlB. 07PF0776 expresses an increased abundance of cell surface-associated InlB in comparison to another commonly studied *L. monocytogenes* isolate, 10403S. This clinical isolate is also found to induce a higher rate of fetal resorption in mice, indicating a possible role for InlB in fetal rejection. In this study we compared the ability of 10403S and 07PF0776 to invade and replicate within the human placental cytotrophoblast cell line HTR8/SVneo and further assessed the dependence of bacterial invasion on InlB. Placental cells were grown in tissue culture dishes and infected with either 10403S or 07PF0776 for one hour, followed by the addition of gentamicin to kill extracellular bacteria. Both isolates were found to be capable of invading HTR8/SVneo cells at a low multiplicity of infection (MOI of 1.5 bacteria per cell). Trophoblasts infected with 07PF0776 exhibited slightly increased levels of bacterial invasion in comparison to cells infected with 10403S; both isolates exhibited similar levels of intracellular replication. Mutant strains lacking InlB exhibited significant reductions in host cell invasion, with 07PF0776 exhibiting an increased dependence on InlB for invasion in comparison to 10403S. Over-expression of InlB increased trophoblast invasion for 10403S by more than fifty-fold. Furthermore, we obtained first trimester primary extravillous trophoblasts and infected them with 10403S, 07PF0776, and 10403S $InlB^{HI}$. We found that the trend is conserved in these first trimester primary cells. Overall, our data indicates that the virulence factor InlB plays a critical role in mediating *L. monocytogenes* invasion of human placental trophoblasts.

XENOPHAGIC CLEARANCE OF LISTERIA MONOCYTOGENES: THE ROLE OF BACTERIAL VIRULENCE FACTORS AND TFEB

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Listeria monocytogenes (Lm), an intracellular pathogen, subverts host immune defenses by manipulating the cytosolic environment. Xenophagy is a selective autophagy pathway, a critical innate immune response, targeting intracellular pathogens for degradation. The recruitment of distinct autophagy proteins is determined by Lm localization: cytosolic or vacuolar. Cytosolic bacteria trigger ubiquitin-dependent recruitment of autophagy adaptors, including p62, facilitating sequestration within xenophagosomes. Conversely, vacuolar bacteria undergo fusion with lysosomal membrane proteins, such as LAMP1. Effective bacterial clearance is dependent upon lysosomal acidification and hydrolytic activity, essential for xenophagosomes maturation. The transcription factor EB (TFEB), a master regulator of autophagy-lysosomal gene expression, orchestrates autophagosomal and lysosomal biogenesis and function, thereby promoting efficient Lm degradation. To elucidate the role of bacterial virulence in xenophagy evasion, we employed a targeted mutational strategy. Our findings demonstrate that specific virulence factor mutations modulate host autophagy responses, influencing autophagy protein recruitment and lysosomal degradation efficiency. This study provides novel mechanistic insights into the intricate interplay between Lm virulence and host xenophagy, suggesting potential therapeutic strategies for enhancing bacterial clearance through autophagy modulation.

CRIF1 GENE THERAPY AMELIORATES INFLAMMATORY BOWEL DISEASE BY SUPPRESSING TH17 CELLS AND FIBROSIS THROUGH MITOCHONDRIAL FUNCTION REGULATION

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Background: CR6-interacting factor 1 (CRIF1) is a nuclear transcriptional regulator and a mitochondrial inner membrane protein. Although serious modifications of the tissue architecture of the small intestine have been reported in CRIF1-deficient mice, how this may affect the development of inflammatory bowel disease (IBD) remains unclear. We investigated the effects of CRIF1 on mice with colitis.
Methods: In DSS-induced colitis mice administered p3XFLAG-CMV-10-CRIF1, clinical symptoms were evaluated. Mitochondrial morphology in the intestinal tissues of colitis mice and UC patients was observed by electron microscopy. Level of CRIF1 in the splenic mitochondria of colitis mice or human PBMCs were investigated by western blot or real-time PCR, and the amount of IL-17 in the supernatant of healthy PBMCs co-cultured with CRIF1-overexpressing mitochondria was investigated by ELISA.
Results: Overexpression of CRIF1 attenuated the severity of colitis, alleviated weight loss, and intestinal shortening. Moreover, overexpression of CRIF1 significantly reduced the levels of proinflammatory and necroptosis-related factors in colon and inhibited intestinal fibrosis. The intestines of these mice showed a reduced level of CRIF1 and altered mitochondrial morphology. Transplantation of CRIF1-overexpressed mitochondria into mice with colitis alleviated disease severity. Patients with ulcerative colitis exhibited decreased CRIF1 levels with dysfunctional mitochondria in inflamed colonic tissue. CRIF1-overexpressing mitochondria inhibited IL-17 production in PBMCs from healthy control.
Conclusion: Our findings demonstrate that CRIF1 alleviates IBD by suppressing inflammation and fibrosis by improving mitochondrial function. Improving mitochondrial function through CRIF1 may be a potential therapeutic strategy for IBD.

BIFIDOBACTERIUM BIFIDUM BGN4 AMELIORATES OSTEOARTHRITIS PROGRESSION BY SUPPRESSING THE EXPRESSION OF INFLAMMATORY MEDIATORS LTB4R AND PGE2

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Osteoarthritis (OA) is a chronic degenerative disease characterized by abnormal bone remodeling, altered synovium structure, and the destruction of joint cartilage. Patients worldwide with OA suffer individual and socioeconomic burdens. Modification of the gut microbiome has been suggested as a new treatment for OA. Bifidobacteria are probiotics that prevent the progression of inflammatory diseases, including inflammatory bowel disease and OA. Here, we investigated the inhibitory effects of Bifidobacteria bifidum BGN4 on the progression of OA using rats with monosodium iodoacetic acid (MIA)-induced OA.

We measured the paw withdrawal latency (PWL) and threshold (PWT) using the von Frey hair assessment procedure and made weight bearing measurements to identify the inhibitory effects of BGN4 on pain. The effects of BGN4 on cartilage were assessed using Safranin O staining of knee tissues. The influence of BGN4 on the expression of inflammatory factors (IL-1β, LTB4r, and PGE2) and enzymes that degrade extracellular matrix (ECM) such as MMP9 and MMP13 was investigated via immunohistochemistry.

BGN4 relieved pain and inhibited the destruction of articular cartilage. BGN4 decreased the expression of inflammatory markers (IL-1β, LTB4r, and PGE2) and MMP9 and MMP13, which are responsible for cartilage destruction.

In conclusion, BGN4 inhibits the progression of OA by suppressing the expression of inflammatory factors and MMPs

LOSS OF O-ANTIGEN DUE TO *WBBL* MUTATIONS IS COMMON AND ASSOCIATED WITH INCREASED MORTALITY IN *ESCHERICHIA COLI* BLOODSTREAM INFECTIONS

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Escherichia coli is the most common cause of bloodstream infections in the western world. *E. coli* inhabits the human gastrointestinal tract where it can exist as a commensal or a pathogen and it is under constant evolutionary pressure from the host, antibiotics and the surrounding microbiota. This work examines how in-host genomic adaptations impact the biology of *E. coli* and influence the interaction with the host immune system. Using serial isolates from six patients with relapsed *E. coli* bacteremia, we demonstrate that disruptive mutations in LPS synthesis genes frequently arise. In two patients, perturbations in LPS synthesis resulted in O-antigen loss and a rough-LPS phenotype. These structural alterations in LPS led to reduced pathogenicity in a murine bacteremia model, increased sensitivity to bile acids, and enhanced complement-mediated killing in human serum. Notably, both isolates with rough-LPS harbored disruptive mutations in *wbbL*, a glycosyltransferase gene essential for synthesis of O25b, the most common O-antigen serotype in the globally disseminated multidrug-resistant sequence type (ST) 131 lineage. To assess the broader relevance of these findings, we screened for mutations in *wbbL* in 61 O25b ST131 bloodstream isolates and found 18% (11/61) carried missense mutations, frameshifts or transposon insertions inactivating *wbbL*. These mutations uniformly eliminated O-antigen production and increased sensitivity to human serum. Clinical outcome analysis revealed patients with bloodstream infections due to *wbbL*-deficient strains had significantly higher mortality rates and an increased incidence of septic shock. This study identifies O-antigen modification as a common consequence of in-host adaptation, resulting in paradoxically decreased laboratory pathogenicity and increased virulence in humans.

ELUCIDATING THE STRUCTURE, BIOSYNTHESIS AND BIOLOGICAL RELEVANCE OF A NOVEL PIGMENT PRODUCED BY STREPTOCOCCUS PYOGENES

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Streptococcus pyogenes (Group A Streptococcus, 'GAS') is a major human-restricted pathogen responsible for a wide range of infections, from mild localized pharyngitis to severe, life-threatening events such as necrotizing fasciitis, and for inducing acute and chronic rheumatological conditions. Despite its well-documented clinical importance and over a century of extensive research, gaps persist in understanding the activities of this pathogen. We report the discovery of a novel pigment produced by Strep A when cultured in a replete, chemically defined medium. Color development accumulates during growth, requires exposure to oxygen, and remains associated with the bacterial cell. Though only 20% of a small strain collection produced the pigment (8 of 40), positive cultures were overrepresented by M1 and M89 serotypes. Pigment production is inhibited when the Rgg2/Rgg3 quorum sensing system is active, though by unknown means. Given that pigments are known virulence determinants in pathogens like Staphylococcus aureus and Streptococcus agalactiae, we hypothesize that the S. pyogenes pigment enhances its virulence and fitness. We seek to understand its biosynthesis, its regulation, and how its production benefits the organism. To identify the metabolic pathway required for biosynthesis, 20,000 transposon mutants were screened for pigment loss in liquid culture. We identified 90 independent hits enriched in pathways associated with isoprenoid biosynthesis, purine biosynthesis, guanosine transport, and mixed acid fermentation. We are actively seeking the pigment's molecular composition by extracting and purifying the compound(s) by HPLC, LC-MS/MS and NMR. As color development requires oxygen, we are testing whether the pigment protects from oxidizing agents and antimicrobial compounds produced by host immune cells. Success in these aims could uncover new targets for therapeutic intervention and advance our understanding of GAS pathogenesis, ultimately contributing to the development of more effective treatments for GAS infections.

DISTINCT MATERNOFETAL IMMUNE SIGNATURES DELINEATE PRETERM BIRTH ONSET FOLLOWING URINARY TRACT INFECTION

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Preterm birth is the leading cause of mortality in infants, resulting in over one million neonatal deaths annually. Maternal urinary tract infection (UTI) during pregnancy increases risk for preterm birth; however, biological processes mediating UTI-associated preterm birth are not well-described. Despite abundant clinical correlations, there is limited data exploring this relationship mechanistically, in part due to lack of animal models that mimic clinical presentation in humans. Taken together, there is an urgent need for improved treatment options and mechanistic insight to factors driving UTI-associated preterm birth. We developed a murine model of UTI-associated preterm birth with uropathogenic E. coli (UPEC), the causative agent of over 70% of UTIs. Mice were infected transurethrally at embryonic day 13.5, then assessed for signs of labor onset 4 hours post-infection. Maternal bladder, lymph nodes, decidua, and placenta were evaluated for transcriptional profiles, cytokine expression, and immune cell infiltration. UTI challenge resulted in preterm birth in about half of dams. Dams experiencing preterm birth displayed excessive bladder neutrophil infiltration, decreased systemic IL-10, and increased uteroplacental Th17/Treg cell ratios compared to non-laboring infected dams, with no differences in bacterial burdens. Live UPEC were recovered from the ileal lymph node, the shared draining lymph node between the bladder and uterus, with a greater proportion of UPEC-positive cells in preterm dams. Furthermore, treatment with Ponesimod, an inhibitor of T cell egress from lymph nodes, reduced Th17/Treg cell ratios and completely prevented preterm birth. We further performed multiplex cytokine urine analyses of a human pregnancy cohort with known birth outcomes. These analyses yielded a non-invasive, highly predictive three-model system for evaluating preterm birth risk implicating T cell-related cytokines. We propose a model whereby excessive maternal bladder inflammation in response to UTI drives immune cell trafficking to the lymph node and subsequent T cell activation at the maternofetal interface, triggering preterm labor. Further, we reveal the potential prognostic value of urine cytokine levels, with known or unknown culture status, for birth outcomes in a human cohort.

DETERMINING THE ROLE OF THE UROPATHOGENIC *ESCHERICHIA COLI* PLASMIDOME IN WITHIN-HOST ADAPTATION TO DIFFERENT ENVIRONMENTS

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Background: It is unknown why the same *Escherichia coli* strains that colonize the gut as commensals cause disease in the urinary tract. Thus far, no genetic signature has been identified for disease-causing uropathogenic *E. coli* (UPEC) strains, making it difficult to predict which strains may cause a urinary tract infection (UTI). We aimed to identify niche-specific genetic signatures in *E. coli* lineages shared between the GI and urinary tracts with the goal of determining which functions mediate within-host adaptation to different body sites.
Methods: Data came from a longitudinally sampled cohort of 127 patients with positive clinically indicated urine cultures at four hospitals in St. Louis, Missouri; Durham, North Carolina; Philadelphia, Pennsylvania; and Chicago, Illinois. Stool and urine isolates were collected from these patients over the six months following UTI occurrence; sampling continued for another six months in the event of a recurrence.
Results: Short read sequencing revealed that the 976 included isolates came from 119 distinct lineages profiled via single-nucleotide polymorphism (SNP) distances based on patient-specific core-genomes. This analysis allowed identification of lineages persisting in the GI and urinary tracts, as well as in both environments. Homogeneous within-habitat mobile genetic element (MGE) carriage was observed in these lineages, indicating that MGEs may drive the niche-specific genomic plasticity of UPEC. Long read sequencing of 285 of these isolates from 20 patients has revealed 474 plasmidic contigs with a variety of replicon types. The gene profiles of these plasmids differ between persister types (p = 1x10-3; PERMANOVA) and based on recurrence status (p = 2x10-3; PERMANOVA), with some plasmid groups being found only in certain persister or recurrence types. Iron acquisition genes like *tonB* are associated with gut persistence (*lrt-p = 1.25x10-6, β = 1.51*), while global transcriptional regulator *hns* is associated with urinary persistence (*lrt-p = 6.43 x10-8, β = 5.28*).
Discussion: These results suggest that plasmidic gene carriage may be the differentiating factor between an *E. coli* lineage's commensalism or pathogenesis.

FROM DNA BINDING TO IMMUNE PROTECTION: EXPLORING THE ROLE OF HU PROTEIN IN *FRANCISELLA TULARENSIS* PATHOGENESIS

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The **HU protein**, a key member of the nucleoid-associated protein family, serves as an important transcription factor in bacteria, including the highly pathogenic *Francisella tularensis*. Typically, HU functions as a DNA sequence–nonspecific binding protein, contributing to DNA winding and the separation of transcriptional units. In this study, we identified potential HU binding sites in *F. tularensis* subsp. *holarctica* FSC200 using ChIP-seq, revealing two possible binding motifs that vary depending on growth conditions. We further demonstrated that the FSC200 HU protein can induce negative DNA supercoiling in the presence of topoisomerase I. Notably, HU was found to interact with the DNA region upstream of the *pigR* gene and within the *clpB* gene, suggesting its role in regulating the expression of PigR and ClpB. Additionally, we identified that arginine 58—and to a lesser extent, arginine 61—are critical for HU's DNA-binding ability, its capacity to introduce negative supercoils, and for maintaining the overall structure and winding of the chromosomal DNA in FSC200. To assess the biological relevance of HU, we introduced mutations at arginine 58, arginine 61, and serine 74. These mutations significantly reduced the virulence of FSC200 both *in vitro* and *in vivo*. Importantly, immunizations with these mutant strains provide up to 100% protection in mice against challenge with the wild-type strain. Overall, our findings enhance the understanding of HU protein's role in *F. tularensis* pathogenesis and highlight its potential as a target for tularemia vaccine development.

IN VITRO CHARACTERIZATION OF SCHUS4ΔCLPB INFECTION FOR MHC IMMUNOPEPTIDOME ANALYSIS AND EARLY IMMUNOPEPTIDOMIC FINDINGS

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Francisella tularensis (*Ft*) is a Gram-negative, facultative intracellular pathogen that causes the zoonotic disease tularemia. In addition to its natural occurrence, it is classified as a potential biothreat due to aerosol transmission and low infectious dose. Current vaccine candidates, such as the live vaccine strain (LVS), offer limited protection, particularly against the highly virulent *Ft* subsp. *tularensis*. A promising alternative under investigation is SchuS4ΔclpB, an attenuated strain derived from subsp. *tularensis*, generated by deletion of the *clpB* gene. This mutant shows reduced virulence and improved protective efficacy in animal models.

In this work, we set up MS-based immunopeptidomics workflow to identify bacterial antigens potentially involved in the vaccine-induced T cell response. Focusing on CD4+ T cells, we aimed to identify *Francisella* peptides presented on MHC class II molecules following infection of bone marrow-derived dendritic cells (BMDCs) with SchuS4ΔclpB. To characterize the infection model, we determined the multiplicity of infection (MOI) and evaluated cell viability, bacterial distribution, intracellular replication, and dendritic cell activation. Cell viability showed a time- and dose-dependent decline, decreasing from 84% at MOI 5 to 67% at MOI 20 and 52% at MOI 80 by 27 hours post-infection. Bacterial distribution at 1 hour post-infection increased with MOI, with 5% of BMDCs infected at MOI 20 and 11% at MOI 80. Intracellular bacterial proliferation, assessed at 2, 6, and 24 hours post-infection, showed a statistically significant increase over time. Flow cytometry analysis showed upregulation of MHC II and CD86 on BMDCs compared to uninfected controls, indicating cellular activation, while CD11c expression remained stable across all conditions.

Further, we performed immunopeptidome analysis to obtain an initial profile of bacterial antigens presented during infection and to determine the optimal bacterial load for infection. BMDCs were infected at MOIs of 5, 20, and 80 for 22 hours, followed by isolation of MHC II peptide complexes and LC-MS/MS analysis of immunopeptides. We have identified thousands of endogenous MHC II peptides across the tested conditions, and importantly, several *Francisella* peptides were hidden in the self-peptidome. This exploratory data will guide the selection of optimal MOI for the next experiments, which will be performed in biological replicates to ensure robustness, reproducibility, and maximal coverage in $CD4^+$ T cell epitope identification.

TOLL-LIKE RECEPTOR-MEDIATED REPRESSION OF VITAMIN D-INDUCED CATHELICIDIN ANTIMICROBIAL PEPTIDE GENE EXPRESSION MAY INVOLVE NF-κB

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Cathelicidin antimicrobial peptide gene, CAMP, defends against a range of bacterial diseases. Vitamin D receptor (VDR) biding to a vitamin D response element in the CAMP gene promoter in the presence of 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] induces expression of the CAMP gene in both humans and primates, particularly in macrophages. To evade immune responses, numerous pathogens have devised ways to decrease the production of CAMP. The activation of toll-like receptors (TLRs) 2, 3, and 4 can suppress 1,25(OH)2D3-induced expression of CAMP in human peripheral blood-derived macrophages by an unknown mechanism. Since macrophages are critical for innate immune responses against a variety of pathogens, we propose that CAMP suppression could affect infectious disease pathology in patients.

We hypothesize that TLR signal transduction pathways via downstream transcription factors are responsible for the inhibition of vitamin D-induced CAMP expression. To identify the mechanism by which TLR signaling suppresses CAMP expression, we implemented a methodical CRISPR-Cas9 gene knockout approach using PMA-differentiated THP1 macrophages, beginning at the cell surface and progressing toward key intracellular mediators. We first targeted TLR4, which responds to LPS and strongly suppresses vitamin D–induced CAMP expression in both primary and PMA-differentiated THP1 macrophages. We then targeted the two primary adaptor proteins, MyD88 and TRIF, of TLR4. Furthermore, we knocked out IRF3, a transcription factor activated by TRIF.

Disruption of TLR4, TRIF, and MyD88 alleviated the suppression, but IRF3 knockouts still displayed suppression of CAMP gene expression in the presence of 1,25(OH)2D3. Taken together, these data suggest that NF-κB may mediate the suppression of vitamin D–induced CAMP expression, because NF-κB is a central transcription factor activated by both MyD88- and TRIF-dependent pathways. It represents a potential point of convergence responsible for the observed suppression. Currently, we are generating NFKB1 knockouts to determine its role in suppression. Upon completion of this work, we expect to show that NFKB1 activation mediates the suppression of 1,25(OH)2D3-induced CAMP gene expression. Future work will focus on determining the mechanism by which this occurs and determining if NFKB activation also explains the suppression we observe in primary macrophages. Our findings will further our understanding of host-pathogen interactions which could inform clinical evaluation and improve outcomes for infectious disease patients.

DIETARY LIPIDS AND MICROBIAL LIGANDS PROMOTE MITOCHONDRIAL DYSFUNCTION AND TYPE I INTERFERON SIGNALING IN MACROPHAGES

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Metabolic disease is a rising concern in numerous populations and results from a combination of dietary and lifestyle factors, including consumption of a low fiber western-style diet (WD) enriched in saturated fat and simple sugar. WD drives metabolic disease via directly impacting energy homeostasis and indirectly by altering the microbiome and promoting chronic inflammation. We previously identified a Mmp12+ macrophage subset responsible for promoting adipose tissue inflammation and systemic insulin resistance in a WD-induced metabolic disease mouse model. We found that a gut pathobiont, Oscillibacter valericigenes, was strongly associated with the Mmp12+ macrophage signature in vivo and induced Mmp12 expression in macrophages in vitro through secretion of TLR2+5 ligands. Next, by leveraging targeted lipidomics data we identified a novel role for the dietary saturated fat, myristic acid, in promoting the inflammatory Mmp12+ macrophage signature. In vitro, macrophages stimulated with both Oscillibacter and myristic acid expressed higher Mmp12 than individual stimulations, indicating an inflammatory interaction effect between dietary lipids and microbiome ligands. To comprehensively assess the macrophage response to these stimuli we employed deep proteomic and transcriptomic analyses across multiple timepoints to construct a multi-omic partially directed covariation network with differentially expressed genes and proteins. Network topology analysis revealed key connections between upregulation of type I interferon signaling and downregulation of mitochondrial metabolism clusters, with most features differentially expressed at early time points. Late cluster responses were dominated by chromatin remodeling and DNA damage repair functions, driven by early metabolic and interferon signaling changes. Furthermore, we identified regulatory candidates in the network generated by Oscillibacter and myristic acid co-stimulation, revealing both chemokines known to promote adipose macrophage inflammation and a novel role for translation regulation in macrophage-intrinsic inflammation. Altogether, using a systems approach, we uncovered how dietary lipids and microbiota-derived signals create dual stressors that amplify cell-intrinsic damage signals to exacerbate macrophage-driven chronic inflammation.
This work was supported by the Division of Intramural Research of NIAID, NIH.

TRANSCRIPTIONAL PROFILING OF *PSEUDOMONAS AERUGINOSA* DURING EPITHELIAL CELL INFECTION REVEALS ZINC LIMITATION IN THE INTRACELLULAR NICHE

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It is now recognized that the gram-negative pathogen *Pseudomonas aeruginosa*, once considered exclusively extracellular, can invade and survive inside epithelial cells. Within this intracellular niche, bacteria exhibit increased tolerance to antibiotics, forming reservoirs that contribute to chronic, treatment-resistant infections. This is particularly concerning given *P. aeruginosa*'s high intrinsic resistance to many antibiotics. To investigate the bacterial adaptations required for intracellular survival, we transcriptionally profiled *P. aeruginosa* within bladder epithelial cells at 2hr, 24hr and 48hr post-infection, using Pathogen Hybrid Capture, a novel enrichment method we previously developed. This approach enables dual transcriptional profiling of host and pathogen from low-input samples. This dataset represents a novel comprehensive characterization of *P. aeruginosa* transcriptional changes within this niche. Temporal analysis revealed nine distinct temporal expression patterns. Notably, genes involved in transcription and translation were significantly downregulated in intracellular bacteria compared to log-phase controls, particularly at later infection time points—suggestive of a persister-like state. Consistent with prior findings, we observed early upregulation of type III secretion system genes, but, surprisingly, we find that these are downregulated at the later time points. A striking finding, that has not been previously reported, was a transcriptional shift related to zinc homeostasis. At later time points, intracellular bacteria downregulated zinc-dependent genes while upregulating zinc-independent paralogs as well as zinc import systems. Functional validation using a gentamicin protection assay showed that mutants lacking two zinc import systems had reduced intracellular survival, underscoring the importance of zinc acquisition during infection. Together, these results demonstrate that *P. aeruginosa* experiences zinc limitation within epithelial cells and must transcriptionally adapt to this environment to survive. Our findings define the transcriptional landscape of intracellular *P. aeruginosa* and highlight zinc homeostasis as a critical factor for survival within this niche. Understanding the pathways essential for intracellular bacterial survival constitute an important step toward treating and preventing chronic infections caused by *P. aeruginosa* and other intracellular bacterial pathogens.

LYSINE DEGRADATION INCREASES ADHERENT AND INVASIVE *ESCHERICHIA COLI* COLONIZATION FITNESS IN THE INFLAMED GUT

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Gastrointestinal (GI) inflammation arises during infectious and non-infectious inflammatory diarrhea. Crohn's disease (CD) is a subset of inflammatory bowel disease that is characterized by patches of inflammation throughout the gastrointestinal tract. CD has an unknown etiology but host genetic factors and the microbiota are thought to contribute to its development. CD patients have an altered gut microbial composition with an increase in Enterobacteriaceae, specifically, adherent and invasive *Escherichia coli* (AIEC). This change in microbial composition has been linked to changes in amino acid availability as patients with CD exhibit increased levels of luminal amino acids. This suggests that AIEC uses these nutrients to enhance colonization during inflammation.

However, there is limited research on how opportunistic microbes utilize amino acids during gut inflammation. Previous research has shown that AIEC changes their metabolism to prioritize amino acid over sugar consumption during colitis. Specifically, AIEC has been shown to use L-serine and L-aspartate during colitis. With the increase availability of amino acids and the prevalence of AIEC in CD patients, it's important to understand how bacteria in non-infectious inflammatory disease respond to environmental nutrients to improve colonization and exacerbate inflammation.

Thus, using a colitis mouse model, I will test whether amino acid utilization impacts AIEC fitness during inflammatory conditions. To address this, we constructed an arrayed library of amino acid transporter-deficient AIEC mutants and tested their colonization in the DSS colitis mouse model. Our findings indicate that the uptake of methionine, lysine, leucine, isoleucine, and valine significantly contributes to AIEC colonization during colitis. Moreover, AIEC uses cadaverine, a lysine metabolite, to counteract reactive oxygen species during murine colitis. These results suggest that the transport and metabolism of lysine are critical for AIEC colonization during episodes of gut inflammation. Identifying the sources of these amino acids could provide valuable insights for developing targeted therapies to mitigate AIEC blooms in inflammatory bowel disease.

A MURINE MODEL OF THE GUT–BLADDER AXIS REVEALS COLONIZATION RESISTANCE AND UPEC PERSISTENCE PATHWAYS

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Urinary tract infections (UTIs) affect over 400 million individuals globally each year, and a particularly debilitating aspect of this disease is its high recurrence rate, with approximately 25% of individuals experiencing repeat infections over time. Uropathogenic *Escherichia coli* (UPEC), responsible for over 75% of UTIs, is often detectable in the gut of affected individuals both during and after UTI, despite antibiotic treatment. To investigate the dynamics of UPEC colonization within the gut, and track recurrence as a function of biogeography and UPEC-microbiome interactions, we developed a novel murine model comprising Enterobacterales-free, as well as mice associated with a commensal *E. coli* strain. Additionally, we eliminated coprophagy by using open-bottom cages, allowing us to dissect bladder-to-gut dynamics without interference from coprophagy. We found that UPEC reaches the gut as early as 24 hours post-UTI and rapidly expands, regardless of the presence of commensal *E. coli*. We see that the commensal *E. coli* protects against gut colonization when UPEC is introduced orally, however this protective effect is partially lost if UPEC colonizes the gut prior to the introduction of the commensal *E. coli*. These observations suggest that a gut-adapted strain from the source patient may serve as a probiotic to outcompete a UPEC strain. Finally, we observed that antibiotic-mediated depletion of gut *E. coli* not only facilitates UPEC colonization from both oral and bladder origins, but also significantly increases the frequency of gut-to-urinary tract transit events and bacteriuria. Metagenomic sequencing of gut microbiota prior to and after antibiotic treatment revealed depletion of species such as Rikenellaceae and Oscillospiraceae, which may contribute to enhanced UPEC colonization or gut-to-bladder transit, along with increases in Mucispirillaceae, Peptococcaceae, Anaeroplasmataceae, and Lactobacillaceae. In total, these findings demonstrate that UPEC can establish stable gut reservoirs following a UTI, with the potential to reseed the bladder and drive recurrence. Our results reveal that the interplay between UPEC and commensal *E. coli* modulates colonization dynamics, and that antibiotic treatment, while intended to resolve infection, may disrupt protective microbiota and promote conditions favorable to recurrent UTI (rUTI). This work emphasizes the critical role of gut ecology in rUTI pathogenesis and highlights the need for microbiome-informed strategies that move beyond antibiotic therapy.

REPURPOSING SULFAPYRIDINE TO TARGET VIRULENCE GENE EXPRESSION IN ENTEROHEMORRHAGIC *E. COLI*

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Enterohemorrhagic *E. coli* (EHEC) is a food-borne pathogen that can cause hemolytic uremic syndrome (HUS), a life-threatening condition. The use of antibiotics for the treatment of EHEC infections is controversial since it often causes an increase in production of Shiga toxin, the main contributor to HUS.

A different approach for the handling of EHEC infection would be to target virulence by hijacking bacterial signaling systems controlling the attachment of bacteria to the intestinal epithelium. The main driver of EHEC's attachment is a type III secretion system encoded on a pathogenicity island called the locus of enterocyte effacement (LEE). This type III secretion system's expression is tightly regulated, a fact that has been exploited for the design of virulence inhibitors[1].

Considering the challenges in developing novel antimicrobial drugs for niche use, we have turned toward re-purposing already FDA-approved molecules as potential virulence modulators by evaluating structural analogues of hits identified through a large chemical library performed in our lab.

In this study, we explore the use of sulfasalazine and its metabolite sulfapyridine as virulence inhibitors for *E. coli* O157:H7. Using an approach combining in vitro tools such as immunoblotting, fluorescent actin staining and RNA sequencing, we were able to show a decrease in transcription and production of proteins encoded on the locus of enterocyte effacement (LEE) pathogenicity island, as well as a diminution of attachment to epithelial cells. Our mechanistic study of the drug also revealed a previously unknown link between EHEC's folate metabolism and virulence gene expression.

This decrease of virulence can also be observed in *Citrobacter rodentium*, a LEE-bearing murine pathogen. We were able to demonstrate that our anti-virulence strategy significantly increases odds of recovery of C3H/HeJ mice challenged with a Shiga toxigenic *C. rodentium* strain.

[1] Rasko et al. "Targeting QseC signaling and virulence for antibiotic development." Science (New York, N.Y.) vol. 321,5892 (2008): 1078-80. doi:10.1126/science.1160354

ASSESSMENT OF HOST IMMUNE RESPONSE TO GENETICALLY RESISTANT AND SUSCEPTIBLE MYCOBACTERIUM TUBERCULOSIS STRAINS IN A RAW264.7 MACROPHAGE MODEL

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Rationale: Poor adherence to treatment for tuberculosis (TB) disease and the rising incidents of drug resistant Mycobacterium tuberculosis strains are factors that negatively influence TB control. The current study was designed to explore some of the key knowledge gaps concerning M. tuberculosis physiology, specifically looking at the host immune response to M. tuberculosis strains in a macrophage model of infection. We hypothesized that the infection of RAW264.7 macrophages with RIF-resistant and susceptible M. tuberculosis strains will induce different host responses reflected by the secretion of cytokines and chemokines. Our objective was to determine the secretion of the cytokines TNF-α, IL-1β, IL-10, IFN-γ, IL-4, IL-6 and IL-12p40 and chemokines GM-CSF, RANTES and MCP-1 in the harvested cell culture supernatants during 24h and 48h of RAW264.7 macrophage infection with K636WT, K636RIF, H37RvWT and H37RvRIF M. tuberculosis strains. Experimental approach: We assessed the host immune response using Luminex and ELISA technologies. Our results revealed no differences in host response to wild type strains, K636WT and H37RvWT M. tuberculosis strains from different genetic backgrounds. In contrast, there were differences in host response to K636WT and K636RIF M. tuberculosis strains in a RAW264.7 macrophage model of infection. This was confirmed by the observed varying secretion levels of cytokines and chemokines (IL-6, IL-12p40 and RANTES) required to mediate M. tuberculosis growth and survival after 24 - 48h of infection. We concluded that the host response is highly pro-inflammatory towards infection with these tested M. tuberculosis strains, as reflected by vast majority of cytokines and chemokines belonging to this group. Our results further demonstrated that rpoB Ser531Leu mutation might have influenced the RAW264.7 macrophages response to infection with K636RIF M. tuberculosis strain. This knowledge accentuates the importance of understanding the mechanisms of pathogenesis, host-pathogen interactions and host response to infection with drug susceptible and resistant M. tuberculosis strains.

THE ROLE OF THE HEME-BINDING PROTEIN, HEMOPEXIN, IN *KLEBSIELLA* PNEUMONIA

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Introduction: Pneumonia is a common and dangerous infection characterized by alveolar filling with inflammatory exudate and, in severe cases, dissemination of pathogens into the bloodstream. Among the microorganisms that cause pneumonia, *Klebsiella*, an aerobic Gram-negative bacillus, is an important cause of hospital-acquired pneumonia. Heme, a molecule composed of a porphyrin ring containing a covalently-bound iron atom, is a component of hemoproteins such as hemoglobin. Bacteria, such as Klebsiella, can utilize heme as a source of iron; in addition, extracellular labile heme is an alarmin and has complex effects on the host. The host scavenges extracellular heme with the acute-phase protein, hemopexin, but the role of hemopexin in pneumonia has not been investigated to date.
Hypothesis: We hypothesize that hemopexin-mediated clearance of heme protects the host during *Klebsiella* pneumonia.
Materials and Methods: We used murine models of pneumonia and bacteremia, induced by intra-pulmonary or intravenous administration of *Klebsiella* pneumoniae in wildtype C57Bl/6 mice and hemopexin-knockout mice. We quantified lung and blood bacterial content by dilution and culture, measured hemopexin transcription by qRT-PCR and protein using a commercial ELISA, quantified labile extracellular heme concentration using a spectroscopic assay and quantified protein hemoglobin using a commercial ELISA. We assessed the growth of *Klebsiella* in vitro on non-nutrient agarose containing mouse plasma with heme or tin protoporphyrin.
Results: During experimental pneumonia, hemopexin was induced in the liver, resulting in increasing concentrations of hemopexin protein in plasma and lower levels in the lungs. As compared to wildtype animals, hemopexin-deficient mice had markedly increased mortality associated with increased bacteremia but not lung bacterial burden. Hemopexin deficiency resulted in increased plasma free heme, cell-free hemoglobin and increased thrombin formation in the blood of infected animals. In vitro, Klebsiella did not form colonies in media containing wildtype plasma unless the media was supplemented by heme. In contrast, hemopexin-deficient plasma supported the formation of *Klebsiella* colonies, and colony formation was abrogated with the reconstitution of hemopexin-deficient plasma with recombinant hemopexin protein.
Conclusion: Our data indicated that hemopexin protects against *Klebsiella* infection by inhibiting bacteremia and hemolysis, and that hemopexin in plasma inhibits bacterial growth by sequestering heme. We speculate that hemopexin prevents a positive feedback loop in which labile heme in plasma leads to thrombosis and subsequent further hemolysis, and that heme serves to promote the growth of the pathogen in the bloodstream.

INVESTIGATING THE ROLE OF RV3839-RV3840 IN THE RESPONSE OF *MYCOBACTERIUM TUBERCULOSIS* TO NITRIC OXIDE AND IRON

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During infection, *Mycobacterium tuberculosis* (Mtb) encounters multiple environmental stressors, including nitric oxide (NO) and iron limitation, and an ability to mount an integrated response is essential for the bacterium's adaptation and continued survival. The NO transcriptional response of Mtb is known to be regulated in part by the DosRS(T) two-component system. However, DosR only regulates a subset (50) of the 200+ genes in the NO regulon. Further, there is significant overlap between NO- and low iron-responsive genes, though how Mtb adapts to these two stressors concurrently is largely unknown. Here, we find that exposure to NO globally augments expression of low iron-responsive genes and vice versa, with a two gene operon, *rv3839-rv3840*, among the most highly upregulated. Deletion of *rv3839-rv3840* resulted in increased growth under prolonged iron limitation and early exit of Mtb from an adaptive state of growth arrest induced upon exposure to NO/low iron. Interestingly, *Δrv3839-rv3840* Mtb were elongated compared to wild type Mtb in NO/low iron conditions, suggesting effects of this operon on cell growth and division under stress conditions. MALDI-TOF and reverse-phase LC-MS revealed accumulation of coproporphyrin III tetramethyl ester (TMC) in Δ*rv3839-rv3840* Mtb under iron limitation. TMC is a precursor molecule in the endogenous Mtb heme biosynthesis pathway, and these results together suggest a role for Rv3839-Rv3840 in regulating heme biosynthesis in Mtb. Current work is focused on understanding the functional role of Rv3839-Rv3840 during infection, and uncovering how the bacterium's adaptation to NO/iron limitation may be mechanistically linked to endogenous heme biosynthesis.

IDENTIFYING ECOLOGICAL DRIVERS OF DYSBIOSIS IN THE FEMALE REPRODUCTIVE TRACT

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The vaginal microbiota is an essential barrier to disease that is compromised in 1/3 of all women, dramatically increasing rates of sexually transmitted disease, cancer, and pregnancy complications. Aerobic vaginitis occurs when enteric opportunists such as *E. coli* subvert the dominance of glycogen fermenting commensals to expand in the vaginal tract and cause severe inflammation and tissue atrophy. Ecological pressures from the host (nutrient availability, oxygen tension, pH etc.) control microbial community composition, but the specific host-derived factors controlling microbiota composition in the vaginal tract are unclear. Risk factors for aerobic vaginitis include hormonal changes during pregnancy, menopause, etc., suggesting that unknown hormone-induced changes in the vaginal microenvironment may favor the growth of facultative anaerobes. In support of this, we and others have observed that only mice in specific reproductive phases (estrus and metestrus) are susceptible to vaginal *E. coli* colonization, and that stimulating estrus in mice with the synthetic estrogen, β-estradiol 17-valerate, drives the expansion of endogenous aerobic flora in the vaginal tract. Here, we use genetically tractable uropathogenic *E. coli* (UPEC) in combination with specialized animal and tissue culture models to identify the specific host and bacterial metabolic pathways that contribute to aerobic vaginitis. Our findings suggest that estrogen-induced metabolic reprogramming of vaginal epithelial cells towards aerobic glycolysis reduces vaginal hypoxia by inhibiting mitochondrial oxidative phosphorylation and allowing oxygen to diffuse into the lumen. We show that subsequent oxygenation of the vaginal tract fuels the expansion of UPEC in a manner dependent on the bacterial cytochrome oxidase, CydA. Overall, results from this study elucidate important mechanistic drivers of vaginal dysbiosis and reveal new therapeutic approaches for bolstering a protective vaginal microbiota community and strengthening this important, yet understudied aspect of women's health.

IS*1* MEDIATED CHROMOSOMAL AMPLIFICATIONS AS AN ADAPTIVE STRATEGY IN *E. COLI* B STRAINS: PMB HETERORESISTANCE AND BEYOND

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Antimicrobial resistance (AMR) is a growing cause of concern worldwide and has resulted in the need for an improved understanding of the mechanisms leading to the emergence of AMR infections. Currently, polymyxins (colistin) are the last line of defense against Gram-negative infections. Polymyxins are positively charged lipopeptides that disrupt Gram-negative membranes via ionic interactions with electronegative LPS. Gram-negative bacteria combat polymyxin's attack by modifying lipid A of the LPS by adding phosphoethanolamine (PEtN) via *eptA* and/or aminoarabinose (L-Ara4N) sugar via the *arn* operon, masking the negative charge and resulting in resistance. AMR is further complicated by heteroresistance (HR), which is defined as the presence of a drug-resistant subpopulation in an otherwise susceptible population. HR results in mischaracterization of resistant/susceptible populations and can cause treatment failures. We aimed to address the cause of polymyxin B (PMB) HR in Gram-negative bacteria. We have identified a mechanism of PMB HR that is dependent on the presence of the *arn* operon, and involves large, unstable, tandem genome amplifications flanked by insertion sequences. Population analysis profile, MIC testing, and measuring the frequency of resistance in isogenic strains of *E. coli* BL21(DE3) with/without genes involved in LPS modifications show that specific LPS modifications play a role in HR. The frequency of resistance of the Δ*arnA* mutant was around 10-fold lower than that of the Δ*eptA* mutant, suggesting that the arn operon is important for the PMB HR phenotype. PMB resistant mutants were derived in a Δ*eptA* background to enrich for *arn* dependent routes of resistance. ESI-MS and TLC data showed increased L-Ara4N modified lipid A in mutant strains. Whole genome sequencing of the mutants revealed the presence of large chromosomal amplifications encompassing the *arn* operon, which were flanked by insertion sequence elements (IS*1*). RNAseq confirmed the increase in the *arn* operon transcripts in the resistant mutants and revealed expression changes in operons residing outside the amplification, implying that heterogeneity in gene expression could be a direct result of IS*1* mediated amplifications. Additionally, passaging these heteroresistant mutants further in higher PMB resulted in increased resistance due loss of heptose sugars from LPS core, a novel chemotype in the context of PMB resistance. Future studies will investigate the impact of these amplifications on gene expression heterogeneity, which could provide a survival advantage to these bacterial populations under stress and look at how these amplifications can act as a steppingstone to fixed mutations for increased resistance.

INVESTIGATING THE DIFFERENTIAL ADAPTATION OF *MYCOBACTERIUM TUBERCULOSIS* TO NEUTROPHIL AND MACROPHAGE ENVIRONMENTS

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Alveolar macrophages (AMs) are the first host cells to encounter *Mycobacterium tuberculosis* (Mtb) after inhalation, but they are incapable of restricting bacterial growth. These AMs eventually move to the lung interstitium, where they die and release Mtb and antigen, leading to the recruitment of other innate immune cells to the lung. Of the recruited innate immune cell populations, neutrophils are the most abundant and most infected cells in the airways of active tuberculosis (TB) patients; additionally, neutrophil accumulation is linked to poor control Mtb infection in mouse models and humans. Despite the evidence that neutrophils are an important intracellular niche for Mtb, the lifestyle of Mtb within neutrophils is unknown. Using SEquening after Amplicon enRiCHment for TB (SEARCH-TB), we profiled the Mtb transcriptome at 4 hours of culturing with murine bone marrow neutrophils, murine *ex vivo* AMs, or media alone. We found that although Mtb replicates and survives in both AMs and neutrophils, the expression profiles allowing for adaptation to these two cell types is distinct. Specifically, in AMs, Mtb upregulates dormancy (DosR), hibernation (zinc-independent ribosomal subunits), and persistence (stringent response) associated pathways. In contrast, in neutrophils, Mtb upregulates macromolecule synthesis pathways as well as nutrient and metal acquisition pathways. Analysis of the differentially expressed sigma factors and transcription factors between Mtb from neutrophil versus AM infection further supports these data. To begin to determine if specific stresses imposed by either AMs or neutrophils are associated with the dichotomous response of Mtb during infection of these two host cell types, we performed bulk RNA sequencing of the infected host cells. We find that Mtb infection of neutrophils induces expression of *Il10*, early markers of NET release, and iron import genes. This environment elicits the expression of metal uptake pathways in Mtb but is still conducive to macromolecule synthesis. In contrast, the inflammatory genes upregulated in infected AMs strongly induce expression of the DosR regulon and stringent response regulated genes in Mtb. These findings shed new light on what stressors Mtb encounters within the host cell and how Mtb differentially adapts to the neutrophil and AM.

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ORNITHINE CATABOLISM PROMOTES *ACINETOBACTER BAUMANNII* COMPETITION WITH THE MICROBIOTA FOR PERSISTENT GUT COLONIZATION

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Antimicrobial resistance is an urgent threat to human health. Asymptomatic colonization is often critical for persistence of antimicrobial-resistant pathogens. Gut colonization by the antimicrobial-resistant priority pathogen *Acinetobacter baumannii* is associated with increased risk of clinical infection. However, ecological factors shaping *A. baumannii* gut colonization have remained unclear. Here we show that *A. baumannii* and other pathogenic *Acinetobacter* evolved to utilize the amino acid ornithine (*astO*), a non-preferred carbon source. *A. baumannii* utilizes ornithine to compete with the resident microbiota and persist in the gut in mice. Supplemental dietary ornithine promotes *A. baumannii* gut colonization over colonization resistance and long-term fecal shedding of *A. baumannii.* By contrast, supplementation of a preferred carbon source—monosodium glutamate (MSG) and histidine—abolishes the requirement for *A. baumannii* ornithine catabolism. The preferred carbon source histidine supplementation directly promotes *A. baumannii* Δ*astO* persistence in the gut by histidine catabolism. Additionally, fecal metagenomics analysis shows an association between diet and *A. baumannii* gut carriage in human infants. Together, these results highlight that evolution of ornithine catabolism allows *A. baumannii* to compete with the microbiota in the gut, a reservoir for pathogen spread.

BACTERIAL LIPOPROTEIN REMODELING: A KEY MEDIATOR OF COPPER RESISTANCE AND IMMUNE EVASION

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Bacteria possess a remarkable and continuously evolving capacity to evade host immune defenses, driving a persistent race between microbial survival strategies and host countermeasures. Among the bacterial arsenal, lipoproteins stand out as essential membrane components present in both Gram-positive and Gram-negative species, playing a pivotal role in immune system detection and activation. Their high abundance and evolutionary conservation render them ideal microbe-associated molecular patterns (MAMPs), readily recognized by Toll-like receptor 2 (TLR2) complexes on immune cells.

Notably, bacterial lipoproteins undergo N-terminal modifications, generating structurally diverse chemotypes that modulate host immune recognition. Our studies have identified a number of lipoprotein-modifying enzymes—Lit, LnsA/B, and Lhat—and are currently focused on elucidating their contributions to immune evasion. We have demonstrated that these enzymatic modifications differentially influence TLR2-mediated signaling, with certain derivatives, such as lyso-lipoproteins produced by Lit, are immune silent.

In parallel, our data suggest that lipoprotein chemotype diversification may also bolster bacterial resistance to copper; a potent antimicrobial effector employed by macrophages to eliminate intracellular pathogens. We discovered plasmid-encoded *lit* gene paralogs (*lit2*) within select *Listeria monocytogenes* strains, co-located with genes implicated in copper resistance. We identified a two-component regulatory system, CopRS, residing on the same plasmid and coordinating the expression of both copper resistance determinants and lipoprotein-modifying enzymes. This represents the first functional link between lipoprotein remodeling and metal resistance.

Furthermore, we show that CopRS integrates environmental signals—specifically those linked to oxygen-controlled electron transport chain activity—to fine-tune the expression of lipoprotein modifiers. This regulatory mechanism highlights its significance in bacterial virulence and survival under host-imposed stress conditions.

Collectively, these findings reveal that lipoprotein modifications serve a dual function: modulating host immune recognition while concurrently enhancing resistance to metal-mediated toxicity, thereby conferring a multifaceted survival advantage during host-pathogen interactions.

ENTEROCOCCUS FAECALIS ACTIVATES ENTEROHEMORRHAGIC E. COLI VIRULENCE BY INCREASING EXTRACELLULAR ADENINE CONCENTRATION

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Enteric microbiota confers numerous advantages to its host, its diverse population allows protection against pathogens and opportunistic bacteria colonization. However, pathogens can regulate their virulence repertoire to adjust to their environment to successfully colonize their host. Here we show that the pathobiont Enterococcus faecalis promotes enterohemorrhagic E. coli (EHEC)'s virulence. EHEC is a foodborne pathogen colonizing the colon and causing bloody diarrhea, and in severe cases can lead to hemolytic uremic syndrome through Shiga toxin production. EHEC employs a molecular syringe referred to as a type three secretion system (T3SS) to translocate effectors that highjack host cell function leading to the formation of attaching and effacing (AE) lesions on enterocytes. We show that metabolically active E. faecalis secrete a metabolite that enhances expression of the EHEC's T3SS and AE lesion formation on cultured epithelial cells, as well as on human colonoids. Targeted and untargeted metabolomics both in vitro and in the presence of colonoids showed an increase in products of the xanthine-hypoxanthine pathway that results in adenine biosynthesis, and in vitro extracellular adenine concentration is increased in EHEC and E. faecalis coculture. We observed that adenine promotes expression of the T3SS. Moreover, comparison of the transcriptomes of EHEC cultivated in presence of E. faecalis also depicts an increase in genes involved in organic transport, more specifically, adeP involved in adenine imports. An adeP knock-out is not responsive to E. faecalis anymore confirming the role of adenine on EHEC virulence expression. By screening a transcriptional regulator knock out library, we identify that adenine effect was relayed through Hha. We identified that adenine was relieving Hha repression on the transcription of the genes encoding the T3SS. We show here that microbiota produced purines (adenine from E. faecalis in this study) can be perceived by pathogen (EHEC) as signals helping them to successfully colonize their host, highlighting the complexity of pathogen-microbiota-host interactions in the gut.

USING AN IMMORTALIZED HUMAN MICROGLIA CELL LINE TO STUDY THE FUNGAL-HOST INTERACTIONS OF THE NEUROTROPIC YEAST *CRYPTOCOCCUS NEOFORMANS*

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Cryptococcus neoformans, the etiological agent of cryptococcal meningitis (CM), is a globally distributed environmental yeast that mainly causes infections in immunocompromised individuals. Particularly in low-resource countries, the mortality rate of CM can reach 81% and accounts for 19% of HIV/AIDS-related deaths each year. Despite this, cryptococcal infections have limited diagnostic and treatment options, largely due to an incomplete understanding of the host-pathogen interactions. In immunocompromised individuals, once inhaled, *C. neoformans* escapes from the lungs and disseminates with predilection for the central nervous system (CNS). Once in the brain, *C. neoformans* interacts with microglia, the tissue-resident macrophages of the CNS. Previous studies indirectly showed that microglia are ineffective at controlling this fungal infection. The mechanisms underlying this fungal survival and proliferation within the CNS, however, remain unclear. Here, we show that primary and immortalized human microglia (C20 cell line) have limited phagocytic activity that is specific to *C. neoformans* and independent of the yeast's viability, secreted factors, and polysaccharide capsule. We also show that human microglia respond to cryptococcal strains differently than alveolar macrophages, the immune cells that *C. neoformans* will encounter when it enters the lungs at early stages of infection. We have performed a screen and identified novel genes that may be responsible for the yeast's ability to specifically evade phagocytosis by microglia, suggesting it is due to several factors including cell size and cell wall structure. Additionally, we show that human microglia are ineffective at killing phagocytosed C. neoformans, probably due in part to the ability of this yeast to disrupt phagosome maturation and induce phagosome membrane damage in these cells. These findings provide us with fundamental knowledge regarding cryptococcal pathogenesis in the CNS, specifically insight into how *C. neoformans* is recognized by microglia under different conditions. These findings also demonstrate the usefulness of C20 cells to further study how this yeast survives and replicates within the CNS environment. Ultimately, future studies using this model can be used for the development of novel diagnostic and treatment therapeutics.

THE UNIQUE *E. COLI* PAST TOXIN DRIVES BOTH PERSISTENCE AND STRESS RESISTANCE IN EXPEC

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Extraintestinal Pathogenic *Escherichia coli* (ExPEC) are versatile pathogens capable of colonizing and causing disease within numerous host environments, including the bloodstream, the central nervous system, and the urinary tract. Infections caused by ExPEC consistently rank among the most costly and common infections on the planet, and are becoming increasingly difficult to treat due to the rise and global dissemination of antibiotic resistant strains. An especially vexing problem with ExPEC is their ability to cause chronic and recurrent infections, even when challenged by antibiotics to which they are sensitive. Based on preliminary work by our group and others, we speculate that the generation of latent antibiotic-recalcitrant bacterial cells known as persisters enables ExPEC to establish long-lived reservoirs within the gut and genitourinary tract that can seed recurrent infections. In previously published work we identified a dual functioning bacterial toxin protein, PasT, that enhances both ExPEC stress resistance and the development of persisters. We report here that the stress resistance capacity of PasT, which we mapped to a C-terminal StART domain, is well conserved across diverse species. In contrast, the persister phenotype is attributable to a smaller N-terminal domain that appears to be unique among PasT homologues within the E. coli lineage based on phylogenetic analyses and functional assays. When overexpressed, *E. coli* PasT acts like a toxin and can arrest bacterial growth in the absence of the anti-toxin protein PasI. The toxic effects of PasT and its ability to promote persister cell development go hand-in-hand and are dependent on two conserved residues within the N-terminal domain. Furthermore, we found that PasT co-precipitates with ribosomal subunit proteins, and that the toxic PasT N-terminus mediates this association. Finally, we show that PasT can markedly enhance the longer-term survival of ExPEC within the urinary tract. In total, this work indicates species-specific roles for PasT variants in stress resistance and persister cell development, and highlights the possibility of targeting PasT as a means to short-circuit cycles of ExPEC persistence and resurgence within the host.

RESPIRATORY PATHOBIONTS FACILITATE REASSORTMENT OF INFLUENZA A VIRUS

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Influenza A virus (IAV) is a leading cause of global morbidity and mortality from annual seasonal epidemics. Additionally, IAV is a persistent pandemic threat due to its capacity to undergo genetic shift from assembly of novel gene combinations resulting from reassortment of multiple IAV strains. Fitness of reassortant viruses varies depending on several factors, but the mechanism of why reassortment occurs is thought to be due to chance infection of the same host cell at the same time by two different viral strains. Recent work demonstrated direct interaction of IAV with several upper respiratory pathobionts: *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Moraxella catarrhalis*, and *Haemophilus influenzae* and showed several viral particles bound at the surface of a single bacterial cell, leading to a hypothesis that bacterial cells could deliver several viral particles to a single host cell, and therefore serve to enhance viral reassortment. A temperature sensitive and oseltamivir resistant IAV strain was generated and was used in co-infection studies with wild type virus in the presence or absence of bacterial cells, with output of temperature resistant oseltamivir resistant IAV used to demonstrate reassortment. In both *in vitro* and murine models, increased reassortment was seen in the presence of S. pneumoniae. To identify if it was the bacterial cells themselves or a product made by bacteria, reassortment was tested following infection with viable or ethanol-fixed S. pneumoniae. Ethanol-fixed bacteria promoted more reassortment than live bacteria, suggesting that a pneumococcal product is in fact detrimental to viral reassortment. To determine the role of bacterial hydrogen peroxide generation and the pneumococcal pore forming toxin, Pneumolysin, reassortment was performed in the presence of live and ethanol fixed *spxB* and *ply* deficient mutants, and with the addition of catalase to neutralize the bacterial H_2O_2 production by SpxB. Reassortment was enhanced with live or dead *spxB* or *ply* mutants, supporting the role of bacterial toxins reducing viral reassortment. As catalase was unable to restore reassortment in the presence of live wild type S. pneumoniae, Ply was further investigated. Ply can serve as a ligand for Toll-like receptor 4. When TLR4 signaling was blocked using the small molecule inhibitor TAK-242, reassortment was enhanced in the presence of wild type S. pneumoniae, supporting a role for TLR4 mediated sensing of pneumolysin reducing reassortment. Taken together, these data suggest a model whereby bacterial cells are capable of delivery of multiple viral particles to the same cell at the same time to enhance reassortment frequency. However, bacterial toxin production can negate this effect. This work demonstrates the complexity of the upper respiratory microbial community and the impact on pathogenesis of co-infecting viruses.

SCAVENGING OF DEAD BACTERIAL REMNANTS IN HOST CYTOSOL BY SELECTIVE AUTOPHAGY

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Cytosolic innate immune surveillance systems deploy multiple strategies to counteract bacterial invasion. One such mechanism studied by our lab involves the ubiquitination of bacterial surface proteins, primarily mediated by the SCF E3 ligase complex. We recently identified that the ubiquitin chains on the bacterial surface are further recognized by an AAA+ ATPase named p97/VCP, which targets ubiquitinated bacteria to extract ubiquitin substrates from the bacterial surface. This activity likely contributes to bacterial destabilization and lysis, neutralizing cytosolic threats. However, the resulting release of bacterial intracellular contents, including DNA and toxins, can initiate secondary danger, triggering host cell damage and inflammation-associated cell death pathways. Therefore, we speculated that the host might engage in some mechanisms to bypass this condition and restore homeostasis. Here, we describe a host-protective selective autophagy mechanism that distinctly clears damaged or disintegrated cytosolic bacterial debris to mitigate these downstream effects. Initially, we observed a pronounced accumulation of cytosolic bacterial debris in host cells infected with Streptococcus pneumoniae. Disruption of autophagy, either pharmacologically with Bafilomycin A1 or genetically via ATG5 deficiency, leads to a substantial increase in cytosolic bacterial remnants. In contrast, proteasome inhibition with MG132 resulted in only a modest accumulation of debris. These findings demonstrated that autophagy, rather than proteasomal degradation, constitutes the principal pathway for the clearance of dead bacterial material from the host cytosol. Mass spectrometry revealed that SpxB, a pyruvate oxidase localized to the inner membrane of Streptococcus pneumoniae, interacts strongly with the autophagy marker LC3, implicating it as a key mediator of selective autophagic clearance. Under normal conditions, SpxB is shielded from the host cytosol; however, upon bacterial membrane rupture mediated by p97, SpxB is exposed to the host cytosol. We further found that SpxB harbors two functional LC3-interacting regions (LIR) motifs that mediate direct interactions with LC3 proteins in autophagosomes, thereby initiating selective autophagy of bacterial debris. Collectively, our findings highlight the role of SpxB as a novel bacterial autophagy receptor that allows the host to selectively scavenge bacterial debris from the cytosol, thereby facilitating autophagic clearance and maintenance of cellular homeostasis.

A TRANSLOCATION-COMPETENT PORE IS REQUIRED FOR *SHIGELLA FLEXNERI* TO ESCAPE FROM THE DOUBLE MEMBRANE VACUOLE DURING INTERCELLULAR SPREAD

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Type 3 secretion systems (T3SSs) enable bacterial virulence by translocating virulence proteins (effectors) into host cells. *Shigella flexneri* require a T3SS to invade and to spread between cells in the colon. In order to spread, *S. flexneri* forms membrane protrusions that push into the adjacent host cell. These protrusions are resolved into double membrane vacuoles (DMVs) that the bacteria quickly escape. The mechanisms required for escape from the DMV are poorly understood, but the T3SS translocon pore protein IpaC is essential. Here, we show IpaC forms a pore that is competent for the translocation of T3SS effectors as bacteria spread between cells. To do so, we used a genetic approach to test mutations of IpaC that disrupt its ability to translocate and to form pores. We show that during spread, IpaC is efficiently inserted into the plasma membrane, the membrane-embedded IpaC forms pore complexes, and the IpaC-dependent pores translocate effectors that are necessary for *S. flexneri* to escape the DMV. We show that T3SS activation is regulated through a distinct mechanism at spread compared to at invasion; activation of T3SS secretion does not require pore formation during spread. We further show that IpaC enables a sequential breakdown of the membranes of the DMV. Thus, we show that a distinct regulation of the T3SS during *S. flexneri* intercellular spread enables the placement of effectors both around *S. flexneri* and across membranes of the DMV. Altogether, this study provides new insights into how *S. flexneri* escapes the DMV.

THE *SHIGELLA FLEXNERI* EFFECTOR IPAH1.4 DEGRADES THE E3 LIGASE RNF213 AND PROTECTS SHIGELLA AGAINST UBIQUITYLATION

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Ubiquitylation is a conserved pathway that is required for the detection and subsequent clearance of infectious bacteria, viruses, and fungi. Given that ubiquitylation is a clear threat to cytosolic pathogens, cytosolic bacteria like *Burkholderia* are resistant to ubiquitylation. We therefore asked whether the professional cytosolic pathogen and causing agent of bacillary dysentery, *Shigella flexneri* could also escape cytosolic ubiquitylation. By using bacterial genetics and cell biology approaches, we uncovered for the first time how *Shigella* counteracts the host ubiquitylation machinery. Mechanistically, we found that *Shigella* secretes the virulence factor IpaH1.4 which triggers the proteasomal degradation of RNF213, an E3 ligase responsible for ubiquitylating multiple pathogens. Indeed, *S. flexneri* mutants lacking IpaH1.4 are coated with ubiquitin and RNF213 in the host cytosol. Moreover, the complementation with IpaH1.4 drastically reduced the ubiquitin and RNF213 coat on a *S.flexneri* mutant lacking IpaH1.4, while the complementation with the catalytically inactive IpaH1.4 reversed the phenotype. We also discovered that the conjugation of linear, lysine-linked ubiquitin and monoubiquitin to bacteria is solely dependent on RNF213 and independent of the E3 ligase LUBAC. Strikingly, we found that ubiquitylation of *S. flexneri* is insufficient to restrict *S.flexneri* bacterial growth. This finding suggests that *S. flexneri* uses additional virulence factors to escape from host defenses that operate downstream from RNF213-driven ubiquitylation. As a whole, we have discovered the first direct inhibitor of RNF213-driven immunity against S.flexneri.

THE ABILITY TO INHIBIT CAS9 IS BROADLY CONSERVED IN ANTI-CRISPR FAMILIES

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Hosts and pathogens can become locked in molecular arms races, in which a host protein evolves to escape being bound by a pathogen effector protein, which then causes the pathogen protein to evolve to restore binding. This pattern of repeated evolution is thought to lead to specificity in host-pathogen protein pairs, as pathogen effector proteins evolve to specifically maintain targeting of their preferred hosts while losing the ability to target homologous proteins from non-hosts. We investigated this paradigm by studying the ability of diverse homologs of anti-CRISPR proteins to repress the Cas9 CRISPR nuclease. We deeply characterized several anti-CRISPR families, including 50 homologs of AcrIIA1, 156 homologs of AcrIIA2, and 86 homologs of AcrIIA4, for their ability to inhibit Cas9 from *Streptococcus pyogenes* and *Staphylococcus aureus*. Unexpectedly, we found that the ability to repress a given Cas9 was generally maintained across entire families of anti-CRISPR proteins, even for homologs with as little as 40% identity. Anti-CRISPR homologs that did not maintain Cas9 repression often had clearly deleterious mutations, indicating they had likely fully lost function rather than undergone a process of gaining specificity. Our results suggest that Cas9 proteins are not engaged in arms races against anti-CRISPR proteins. This could be because anti-CRISPR proteins often target highly functionally constrained components of Cas9, such as PAM recognition. Instead, bacteria may employ alternative methods to counter anti-CRISPRs; for instance, recent work has found that some bacteria repress anti-CRISPR expression or hyperactivate CRISPR in the presence of anti-CRISPRs. Our work demonstrates the power of comprehensively characterizing the function of homologs to clarify important evolutionary dynamics between hosts and pathogens.

GENOMIC, EPIDEMIOLOGICAL INVESTIGATION OF NURSING HOMES RESIDENTS TO DETECT INDOLENT SKIN COLONIZATION AND TRANSMISSION OF MULTI-DRUG RESISTANT ORGANISMS

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Antimicrobial resistance is a public health threat associated with increased morbidity, mortality, and financial burden. While transmission of multidrug-resistant organisms (MDROs) is tracked in hospitals, nursing homes often have limited infection control surveillance and frequently have higher rates of MDRO colonization. As vital components of healthcare ecosystems, nursing homes (NHs) remain underrecognized reservoirs for MDROs.

Embedded within an epidemiologic study, we conducted skin sampling for both cultivation with subsequent isolate whole-genome sequencing (n=27) and shotgun metagenomic sequencing (n=244) to investigate MDRO colonization dynamics of 38 residents across 15 NHs in California, USA. Our metagenome-assembled genome (MAG) and cultured isolate genome analyses revealed widespread colonization by extended-spectrum β-lactamase (ESBL)-producing Escherichia coli sequence type (ST)131 with antibiotic-resistant E. coli ST93 as an emerging threat. Also detected were vancomycin-resistant Enterococcus faecalis ST109 and ST778, Staphylococcus epidermidis ST2, Proteus mirabilis, Providencia stuartii, and Pseudomonas aeruginosa. Core-genome SNP and phylogenetic analyses revealed clonal dissemination of E. coli ST93 and S. epidermidis ST2 both within and between facilities. Persist detection of E. coli ST131, ST93, and S. epidermidis ST2 on residents skin was observed even with bathing. The skin microbiome of NH residents comprises a broad antibiotic resistome, including cephalosporin, aminoglycoside, fluoroquinolone, macrolide, phenicol, sulfonamide and tetracycline resistance genes. Integrated metagenomic and culture-based approaches are valuable tools for tracking and controlling antimicrobial resistance in nursing homes, where aging populations and inter-facility connectivity pose growing public health challenges.

HOST-DRIVEN K11/K48 BRANCHED UBIQUITINATION ACCELERATES INTRACELLULAR BACTERIAL CLEARANCE THROUGH RAPID VCP/P97 RECRUITMENT

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Microbial invasion of host cells activates immune surveillance through detection of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), leading to rapid immune signalling and pathogen clearance. Ubiquitination has emerged as a crucial strategy for intracellular detection and elimination of invasive pathogens. In this study, we demonstrate that the host utilizes K48- and K11-linked polyubiquitin chains to modify Streptococcus pneumoniae surface proteins as part of innate defense. We further depict that both these ubiquitination events occur simultaneously on one of the most abundant and well-characterised pneumococcal membrane-bound proteins, PspC (also known as CbpA), upon recognition of its degron motif. Bioinformatics predictions supported by biochemical and colocalization analyses identify the host E3 ligase Anaphase Promoting Complex/Cyclosome (APC/C) complex as a mediator of PspC ubiquitination. Consistent with known APC/C substrates, PspC also gets modified with K11/K48 branched ubiquitin chains with the help of the associated E2 enzyme UBE2S. Branched ubiquitin chains facilitate rapid recruitment of host AAA-ATPase VCP/p97 to bacterial substrates, thereby accelerating its clearance relative to substrates modified with homotypic K48 ubiquitin chains. This study highlights the critical role of the ubiquitin-proteasome system in maintaining cytosolic sterility and emphasizes the importance of branched ubiquitination as a potent arm of cell-autonomous immunity.

INVESTIGATING THE ROLE OF AMYLOID-β IN UPEC DRIVEN URINARY TRACT INFECTION

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Initially identified as a key driver of Alzheimer's disease pathology, amyloid-β (Aβ) has recently emerged as an antimicrobial peptide produced in response to infection. Aβ is a pleiotropic innate immune effector that may both interact directly with the pathogen and contribute to downstream inflammatory damage. Uropathogenic *Escherichia coli* (UPEC) is the prominent causative agent of urinary tract infection (UTI) and was therefore used for these studies. To explore the mechanism by which Aβ may be directly interacting with UPEC, we conducted *in vitro* phenotype experiments to measure altered bacterial surface structures. Specifically, Aβ 40 induced bacterial aggregation at 1 μM. We then employed an ascending UTI model in CBA/J mice, in which pathogenic bacteria cause cystitis, pyelonephritis, and urosepsis. In UPEC-infected compared to PBS mock-infected mice, we observed significant Aβ 40 accumulation in the kidneys within 48 hours, detected via ELISA, with a mean difference of 246.2 pg/ml (80.1% increase compared to control). The amount of isoform Aβ 40 had a direct positive correlation to bacterial burden (R=0.84), suggesting that infection severity elicits more Aβ. To assess the translational relevance of Aβ during infection, we analyzed plasma samples from UTI patients and found elevated Aβ levels in UTI-positive individuals (n=36) compared to UTI-negative controls (n=41). Notably, the median Aβ 40 levels differed by 63.6 pg/ml (20.5% increase). Our integrated clinical, animal, and *in vitro* models highlight Aβ's critical role as a key player in the innate immune response to UTI. Future studies will expand patient sample sizes and further elucidate the mechanisms by which Aβ interacts with UPEC, using a range of urovirulence and gene expression assays.

DISRUPTION OF INTESTINAL EPITHELIAL HOMEOSTASIS BY ENTEROTOXIGENIC *E. COLI* (ETEC) HEAT-LABILE TOXIN THROUGH WNT/B-CATENIN ACTIVATION

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Enterotoxigenic *Escherichia coli* (ETEC), an *E. coli* pathovar producing heat-labile (LT) and/or heat-stable (ST) toxins, is a major cause of diarrheal illness in children from low- and middle-income countries. Numerous studies have linked ETEC infection to long-term effects, including growth faltering and malnutrition in children living in these regions. While the canonical mechanisms of ETEC-induced secretory diarrhea are established, the cellular and molecular basis of these non-diarrheal sequelae remains poorly defined.

Here, we show that LT activates WNT/β-catenin signaling independently of WNT ligands, resulting in altered epithelial proliferation and cell-type composition. Using human ileal organoids, single-cell RNA sequencing (scRNA-seq), luciferase reporter assays, and confocal imaging, we identified multiple levels of LT-mediated WNT pathway activation. LT treatment upregulated canonical WNT target genes (e.g., AXIN2, MYC, SOX9, LGR5), as well as genes involved in differentiation (ATOH1, XBP1, OLFM4) and progenitor maintenance (CD24, PROM1), based on bioinformatic analysis of bulk RNA-seq data. To confirm functional activation, we used ileal enteroids transduced with a 7TFP TCF/LEF luciferase reporter. LT treatment significantly increased TCF/LEF-driven transcription, consistent with nuclear β-catenin engagement. Western blot and confocal microscopy revealed increased cytosolic and nuclear β-catenin, including elevated phospho-Ser675 β-catenin and phospho-Ser9 GSK3β, both of which promote β-catenin stabilization and nuclear accumulation. Phenotypically, LT induced marked epithelial hyperproliferation. scRNA-seq revealed upregulation of cell cycle markers (MKI67, CD24, OLFM4) and a shift toward the G2/M phase, with decreased G1-phase cells. These transcriptional changes were confirmed by increased EdU incorporation and MKI67 protein expression, indicating enhanced mitotic activity. LT also altered epithelial cell-type composition. Unsupervised clustering of scRNA-seq data showed expansion of isthmus-like (transit-amplifying) progenitors and reduction of mature enterocytes and secretory cells. LT-treated enteroids also developed a distinct proliferative cluster absent in controls, suggesting disrupted differentiation and impaired epithelial maturation.

These findings demonstrate that LT activates β-catenin signaling and alters intestinal epithelial dynamics. While our data support a mechanistic link between β-catenin activation and impaired epithelial homeostasis, further functional studies are needed to directly establish causality. These results provide insight into how ETEC may contribute to long-term gut dysfunction and stunting beyond acute diarrhea.

QUANTIFYING COUGH, AEROSOLIZATION, AND TRANSMISSION OF *MYCOBACTERIUM TUBERCULOSIS* IN A GUINEA PIG MODEL

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Cough is a hallmark symptom of pulmonary tuberculosis (TB) and a central driver of Mycobacterium tuberculosis (Mtb) transmission. Yet, the mechanisms underlying Mtb-induced cough and the production and dispersal of infectious aerosols remain poorly defined. We developed a modern, environmentally controlled, BSL3-compliant experimental toolbox to quantify respiratory parameters, cough frequency, airborne Mtb particle production, and transmission efficiency in guinea pigs, a physiologically relevant host that mirrors key features of human TB, including granulomatous pathology and a conserved cough reflex.

While guinea pig-to-guinea pig transmission of Mtb was first described by Robert Koch and later studied in detail by Perla and Lurie in the early 20th century, such investigations were largely abandoned before the development of modern biosafety practices. Using our updated platform, we demonstrate that Mtb-infected guinea pigs produce aerosols containing viable bacilli and can transmit infection to naïve animals under controlled conditions by measuring both cell-mediated and humoral immune responses in naive animals. We also demonstrate that airflow dynamics critically shape the likelihood of transmission: high-airflow (well-ventilated) conditions reduce airborne particle burden and completely abrogate transmission.

Our experimental toolbox provides the first quantitative, biosafety-compliant framework for dissecting host, microbial, and environmental factors that drive TB transmission. In addition to recapitulating the biology of natural transmission, it allows real-time measurement of aerosolized Mtb and controlled manipulation of infection parameters such as Mtb lineage, drug resistance and production of nociceptive molecules that trigger cough. These advances open the door to testing the impact of antibiotic regimens, host-directed therapies, or environmental interventions aimed at interrupting the TB transmission cascade.

INVESTIGATING THE EFFECT OF PRETERM GUT MICROBES ON EARLY LIFE COLONIC IMMUNE DEVELOPMENT

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Background: Preterm infants exhibit altered gut microbiome development, experiencing decreased gut microbial diversity and having a gut microbiome dominated by *Escherichia coli*, *Enterococcus* spp. and *Klebsiella* spp. that have the potential to cause bloodstream infections and sepsis. Immune development in early life is shaped by exposure to gut microbes, and preterm infants show abnormalities in immune development during the first months of life including increased inflammatory cytokines and activated T cells. However, the effect that taxa common in the preterm gut microbiome have on immune development is unknown.
Methods: We colonized germ-free dams with microbial isolates obtained from preterm infant stool samples, either as monocolonizations or representative consortia. We sacrificed pups from these dams at DOL14 and DOL28 and collected samples from spleens, peripheral blood, small intestines and colons to perform flow cytometry analysis of immune cell populations. We collected stool samples for metagenomic sequencing to confirm colonizations.
Results: We found an increased percentage of neutrophils in the colon ($p<0.05$) and spleen ($p<0.05$) of mice colonized with *Klebsiella pneumoniae* at DOL14, making up 4.5% of CD45+ cells in the colon and 4% of CD45+ cells in the spleen. At DOL28, we found that *Enterobacter cloacae* monocolonizations have increased colonic B cells composing 53% of CD45+ cells relative to *K. pneumoniae* and *E. coli* at 6.7% and 11% of CD45+ cells respectively. We found that in the small intestine *E. cloacae* monocolonizations show increased CD4 T cells at 26% of CD3+ cells ($p<0.05$) and decreased CD8 T cells ($p<0.05$) at 38% of CD3+ cells. We also found differences in colonic T regulatory cells, with *E. cloacae* monocolonizations showing a decreased percentage of Foxp3+ CD4 T cells at 11.4% of CD4+ T cells, compared to 26.9% and 27.6% for *K. pneumoniae* and *E. coli* monocolonizations respectively.
Conclusions: We found that different key members of the preterm gut microbiota have variable effects on neutrophil, B cell and T cell populations in the colon and small intestine. These results improve our understanding of how altered gut microbiome composition leads to altered immune development in premature infants.

PATHOGENESIS CONTINUUM: LUXS/AI-2 SIGNALING REGULATES CRITICAL GENE TARGETS TO ASSIST IN MULTIPLE STAGES OF *SALMONELLA* INFECTION IN THE HOST

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Salmonella quorum sensing involves producing and detecting Autoinducer-2 (AI-2) signal molecules, which allows it to adapt to its environment and regulate virulence factors, biofilm formation, and other behaviours critical for survival and infection. Understanding these mechanisms is essential for developing strategies to intervene with *Salmonella*'s ability to cause disease. Our study elucidates how AI-2 assists in the multiple stages of *Salmonella* pathogenesis by regulating chemotaxis, adhesion, invasion, and intracellular survival. The chemotactic movement of bacteria is defined by their motility and flagellar movement. In our study, we determined that in quorum-sensing knock-out strains, STM Δ*luxS*, Δ*lsrB*, and Δ*lsrK*, the swimming and swarming motility were abrogated with underexpression of the flagellar genes (*fliC*, *fljB*, *flhD*, and *fliA*) and chemotaxis-related genes (*fliM*, *cheY*, and *motA*). Moreover, we noted minimal transmigration activity of STM in the absence of AI-2 signaling and sensing. We further observed that the knockout strains are attenuated in adhesion and invasion into the Caco-2 epithelial cells and showed reduced invasion into the intestinal epithelial cells of C57BL/6 mice. Additionally, the expression of SPI-1 encoded genes *hilD* (induces chemotaxis and regulator of SPI-1 genes), *hilA*, and *invF* was downregulated in the knockout strains, highlighting the complex regulatory network in *Salmonella* by AI-2 via the HilD axis governing motility and invasion. Interestingly, RNA sequencing revealed that more than 4500 variable genes, including chemotaxis, flagellar structure, and virulence genes, were altered in the knockout strains. Besides aiding in the invasion, this signaling also regulates antimicrobial peptide resistance and intracellular survival in epithelial cells by regulating the *pmrD/AB* system. While regulating the pH-sensing two-component system *phoP/phoQ* mediated SPI-2 master regulators *ssrA/ssrB* and SPI-2 effectors facilitating survival within the acidic *Salmonella*-containing vacuole (SCV) of macrophages. Mechanistically, we elucidate that LsrR, a negative transcriptional regulator of LuxS/AI-2 signaling, shows an unconventional binding interaction with the *phoP* promoter and modulates the mRNA expression. Lastly, using a mouse model, we show that AI-2 signaling is critical for organ colonization and virulence of *Salmonella*, and inhibition of LuxS/AI-2 signaling with a combination of antibiotics can be a novel therapeutic approach. Thus, our study unravels mechanistically how quorum sensing aids in the complete pathogenic process of *Salmonella*.

GABA CONSUMPTION PROMOTES *SALMONELLA* TYPHIMURIUM COMPETITION WITH COMMENSAL *ESCHERICHIA COLI* IN THE INFLAMED GUT

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The host gastrointestinal tract harbors a diverse microbial community that mediates protection against enteric pathogen colonization, a process known as colonization resistance. Enteric pathogens like *Salmonella enterica* serovar Typhimurium have evolved to use their virulence to trigger intestinal inflammation and compete with the gut microbiota by mechanisms that are not fully elucidated.

A comparative genome analysis identified several genes that are intact in *Salmonella* serovars associated with intestinal disease but disrupted in serovars exclusively associated with extraintestinal disease, pointing to their possible role in enteric colonization. These genes include *gabTP*, which encode proteins for the uptake and catabolism of γ-aminobutyric acid (GABA), an inhibitory neurotransmitter that can be produced by both the host and the gut microbiota. We found that mice infected with the *S.* Typhimurium *gabTP* mutant had higher fecal GABA levels than those infected with *S.* Typhimurium wild-type, indicating *gabTP*-dependent GABA depletion during infection. Mice infected with *S.* Typhimurium wild-type also exhibited higher *Il22* transcription levels compared to those infected with the *gabTP* mutant. The cytokine IL-22 induces the production of antimicrobial proteins to which *S.* Typhimurium is largely resistant. Therefore, induction of IL-22 enables *S.* Typhimurium to outcompete commensal *E. coli* in the inflamed gut. We thus hypothesized that *gabTP*-mediated GABA depletion elevates IL-22 during infection, enhancing *S.* Typhimurium competitive advantage over commensal *E. coli*. Mice infected with *S.* Typhimurium wild-type exhibited high levels of IL-22 production by group 3 innate lymphoid cells (ILC3s), a key IL-22 source in the gut, and outcompeted commensal *E. coli*. In contrast, infection with the *gabTP* mutant resulted in lower IL-22 production by ILC3s, and failed to outcompete *E. coli*. Administration of GABA in the drinking water impaired IL-22 production by ILC3s, eliminating the competitive advantage of *S.* Typhimurium wild-type over commensal *E. coli*. Collectively, these results suggest that GABA catabolism by *S.* Typhimurium modulates ILC3-mediated IL-22 production, thereby enabling the pathogen to outcompete commensal microbes.

FUNCTIONAL GENOMIC SCREENING REVEALS HOST-MEDIATED MATURATION OF A RICKETTSIAL SURFACE VIRULENCE FACTOR

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The *Rickettsia* genus contains numerous vector-borne human pathogens of increasing public health concern. Due to their obligate intracellular nature and highly specialized adaptations to host cytoplasmic niches, *Rickettsia* spp. pathogens are also useful models for discovering novel host–pathogen interactions, but are highly understudied largely due to their genetic intractability. Here, using the model rickettsial pathogen *Rickettsia parkeri*, we conducted a flow cytometry-based genome-scale CRISPR/Cas12a knockout infection screen to identify host determinants of *R. parkeri* burden in human cells. Validated hits comprised diverse host pathways such as lipid metabolism and histone modification, unveiling numerous novel host regulators of rickettsial infection for future investigation. Unexpectedly, we discovered that a top screen hit, the host peptidyl-prolyl isomerase cyclophilin A (PPIA/CypA), was required for the formation of the *R. parkeri* actin tails that enable pathogen intracytosolic motility and intercellular spread. Chemical perturbation of PPIA activity rapidly and reversibly blocked *R. parkeri* actin tail formation. PPIA localized to actin-associated *R. parkeri* poles during infection, and strikingly, was specifically required for surface exposure of Sca2, the autotransporter primarily responsible for *R. parkeri* actin tail nucleation. Structural modeling and pulldowns revealed that PPIA directly binds a domain in Sca2 predicted to play a critical role in autotransporter biogenesis. We propose that host-derived PPIA enables Sca2 surface maturation during *R. parkeri* infection through a direct interkingdom protein folding event. As autotransporters are common bacterial fitness or virulence determinants, host-dependent maturation of these surface factors may represent an unexplored axis of the cytoplasmic pathogen–host interface. Our work highlights the potential of host-directed perturbation approaches as a lens through which to explore obligate intracellular bacterial biology.

TREATMENT OF ANTIBIOTIC-RESISTANT *KLEBSIELLA PNEUMONIAE* BY A MULTI-SPECIFIC ANTIBODY AGAINST THE LIPOPOLYSACCHARIDE O-ANTIGEN

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Klebsiella pneumoniae is a serious global threat and accounts for over ten percent of all deaths from bacterial infections. It is a major causal pathogen of lower respiratory tract infections, intra-abdominal infections, bloodstream infections, and complicated urinary tract infections. Much of this mortality is associated with increasing rates of antimicrobial resistance, including to antibiotics of last resort like carbapenems. Multidrug resistant strains have few treatment options and new therapeutics are desperately needed. Monoclonal antibodies offer an attractive alternative to traditional antibiotics based on their superb specificity, potency, and distinct mechanism of action. Characterization of over 10,000 global contemporary clinical isolates suggest that targeting up to the four most common O-antigen serotypes of *K. pneumoniae* lipopolysaccharide (LPS), the primary Gram-negative outer membrane glycolipid, may afford coverage of greater than 90% of infections. To this end, we have isolated and developed four human monoclonal antibodies targeting the O-antigen of serotypes O1, O2, O3, and O4. These mAbs mediate bacterial clearance via opsonophagocytic killing (OPK) *in vitro* and protect against *K. pneumoniae* induced bacteremia and pneumonia in mouse models of infection. A multi-specific mAb combining the O1 and O2 specificities into a single molecule provides cross-serotype activity and synergistic protection when combined with meropenem against carbapenem-resistant *K. pneumoniae* strains. Antibodies targeting all four O-antigens will ultimately be combined into two muti-specific antibodies. Together, our data suggest that a multi-specific mAb may provide broad protection in adjunctive therapy against antibiotic-resistant *K. pneumoniae* infections.

DEVELOPING A BACTERIAL RNA SEQUENCING APPROACH TO STUDY *YERSINIA PSEUDOTUBERCULOSIS* ANTIBIOTIC PERSISTENCE IN A MOUSE MODEL OF SYSTEMIC INFECTION

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Systemic bacterial infections are a major cause of morbidity and mortality globally. One barrier to successful treatment of these infections is decreased antibiotic susceptibility, which may include antibiotic persistence. Antibiotic persistence is a term that describes phenotypic changes that occur in a subpopulation of bacteria, which allows otherwise susceptible bacteria to survive antibiotic exposure. In these cases, antibiotics may be effective in reducing the bacterial load, but once antibiotic levels wane, the surviving bacteria can resume growth and cause relapsing infections. While mechanisms that promote antibiotic persistence have been described in culture, the development of new therapeutic approaches to specifically target bacterial persisters is currently hindered by our limited understanding of antibiotic persistence during infection. We are developing a bacterial RNA sequencing approach to study antibiotic persistence in a mouse model of prolonged antibiotic treatment of systemic infection. In our model, mice are inoculated intravenously with *Yersinia pseudotuberculosis* (Yptb). Mice then receive three doses of doxycycline intraperitoneally. During systemic infection, Yptb colonizes deep tissue sites including the spleen, and forms microcolonies which serve as a model for studying phenotypic heterogeneity and antibiotic persistence. Mouse spleens are collected at defined timepoints to quantify CFUs, visualize Yptb microcolony morphology, and prepare samples for bacterial fluorescence-activated cell sorting and RNA sequencing. We hypothesize that bacterial cells pre-exposed to higher levels of stress will preferentially survive antibiotic treatment due to reduced metabolic activity and growth. Based on prior work, we anticipate specific stress response pathways will promote bacterial survival, including nitric oxide detoxification and DNA damage repair. By collecting bacterial cells from host tissue, we will identify bacterial genes and pathways associated with decreased susceptibility to antibiotics. Additionally, by collecting pools of lower cell numbers for RNA sequencing in combination with RNA FISH, we plan to interrogate the heterogeneity and spatial organization of differential gene expression dynamics within these populations. We are currently completing bacterial cell sorts from host tissues and optimizing RNA FISH protocols. Ultimately, by identifying the mechanisms that promote antibiotic persistence during infection, we will uncover new potential therapeutic targets and strategies to improve treatment efficacy.

SERINE/THREONINE PROTEIN KINASE-TWO-COMPONENT SYSTEM INTERPLAY REGULATES TRANSCRIPTION AND STRESS RESPONSE IN *MYCOBACTERIUM TUBERCULOSIS*.

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Successful host colonization by bacterial pathogens requires appropriate response and adaptation to environmental signals encountered during infection. Two key signal transduction regulatory mechanisms utilized by bacteria for this purpose are serine/threonine protein kinases (STPKs) and two-component systems (TCSs). TCSs are ubiquitous in bacterial species and extensively studied, while the presence and role of STPKs in bacterial biology is becoming increasingly appreciated. *Mycobacterium tuberculosis* (Mtb) possesses similar numbers of STPKs (11) and TCSs (12), but if and how these two regulatory systems coordinate to enable Mtb adaptation in response to key environmental cues such as nitric oxide (NO) and hypoxia remains poorly understood. Mycobacterial protein fragment complementation assays revealed specific STPK-TCS interactions, with a subset of STPKs demonstrating interactions with multiple TCS response regulators. Biochemical assays utilizing purified proteins demonstrated that STPK phosphorylation of DosR, the response regulator of the NO/hypoxia-responsive TCS DosRS, decreased target promoter DNA binding and also steady-state transcription. This excitingly contrasted with increased target promoter DNA binding and steady-state transcription upon phosphotransfer to DosR by its cognate histidine kinase. Finally, we found that a ΔSTPK Mtb mutant exhibited increased DosR regulon transcription at lower NO levels than wild type Mtb, illustrating how STPK phosphorylation of a TCS RR can act to restrain its activation to ensure response initiation only when appropriate. Combined, our work supports the extensive relationship between STPKs and TCSs, and sheds light on the mechanisms underpinning STPK-TCS interplay.

DOES COAGULOPATHY CONTRIBUTE TO THE OUTCOME OF INVASIVE PULMONARY ASPERGILLOSIS?

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Introduction: Invasive pulmonary aspergillosis is a deadly disease caused by the opportunistic mold Aspergillus. As the mold grows in the lungs, fungal hyphae penetrate the epithelium, resulting in lung injury and hemorrhage. We previously reported that this hemorrhage and the consequent release of extracellular heme accelerates fungal growth and worsens the outcome of the infection. However, the host response and attempts to control hemorrhage during aspergillosis remain understudied. We hypothesize that the hemostatic system is protective in host defense during invasive aspergillosis.
Methods: C57Bl/6J mice were partially neutrophil-depleted using a monoclonal antibody, and challenged with Aspergillus conidia via the trachea. We performed serial thromboelastography on the blood of infected mice and control mice, sampled the alveolar lumen by bronchoalveolar lavage (BAL), and assessed lung fungal burden. We also performed ELISAs for coagulation factor Xa and Thrombin-antithrombin complex on BAL fluid samples. We used mathematical modeling to map coagulation factors to thromboelastography curves and predict coagulation factors that explain observed TEG patterns. In some experiments, infected mice were treated with clinical drugs that inhibit factor Xa (apixaban) and prevent fibrinolysis (tranexamic acid), and compared them to controls.
Results: Infected mice had higher levels of factor Xa and thrombin-antithrombin complex in BAL, as well as higher maximum amplitudes in thromboelastography as compared to uninfected mice, indicating appropriate activation of the coagulation cascade. Unexpectedly, infected animals had an elongated time to clot formation on thromboelastography, suggesting coagulopathy. Our mathematical model predicted a potential depletion of factors X or VII. We found a partial depletion of factor VII but not factor X in the blood during infection. Treatment with apixaban increased fungal burden. Treatment with the anti-fibrinolytic drug tranexamic acid resulted in a pronounced reduction in fungal burden in female mice but had no effect on male mice.
Conclusions: Our preliminary studies suggest that coagulopathy is an important component of invasive aspergillosis and that treatment with anticoagulants during infection might lead to worse outcomes in mice. Treatment with an anti-fibrinolytic agent led to a lowered fungal burden in female mice, which suggests that the fibrinolytic pathway is dysregulated during infection, and that agents that aid in clot formation might further improve outcomes in mice.

Acknowledgements: University of Florida's Research Opportunity Seed Fund, NIAID K25AI175668, NHLBI R01HL169974

IMMUNE RESPONSE TO CHLAMYDIA IN SLEEP-DEPRIVED MICE IS RESPONSIBLE FOR THE PATHOLOGICAL OUTCOME OF THE DISEASE

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Chlamydia trachomatis is the most common sexually transmitted infection, which leads to pelvic inflammatory disease, salpingitis, and infertility in women. Previous studies from our research team have shown that circadian factors—such as the time of day of infection and disruptions to the circadian rhythm—significantly affect disease progression and reproductive tract pathology in a mouse model. However, the specific impact of sleep itself, independent of these circadian influences, on Chlamydia pathogenesis remains poorly understood. The current study aims to investigate the effects of sleep deprivation on Chlamydia pathogenesis and the resulting genital tract pathology. Using a sleep-deprived mouse model, we found that mice infected with Chlamydia under sleep-deprived conditions exhibited more severe genital tract pathology compared to non-sleep-deprived infected mice. These findings suggest that sleep plays a protective role against the severe outcomes of chlamydial disease. To understand the processes responsible for the increased pathology in the sleep-deprived group, we measured cytokine and antibody levels. We observed that inflammatory cytokine levels were significantly elevated in the infected sleep-deprived mice compared to the infected control mice. Additionally, Chlamydia-infected control mice had higher levels of mucosal and systemic antibody responses compared to infected sleep-deprived mice. This indicates that sleep deprivation may impair the development of a robust adaptive immune response while exacerbating pathology through a cytokine storm. This research underscores the importance of sleep as a significant immunomodulatory factor in host-pathogen interactions and highlights potential implications for public health interventions aimed at vulnerable populations experiencing sleep deprivation.

UNCOVERING STRAIN-SPECIFIC INNATE IMMUNE SIGNATURES DURING *MYCOBACTERIUM ABSCESSUS* INFECTION

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Mycobacterium abscessus (MAB) is an opportunistic pathogen with remarkable antibiotic resistance and increasing incidence globally, primarily affecting vulnerable populations with underlying lung conditions like cystic fibrosis. While MAB causes severe lung disease, our understanding of host-pathogen interactions in the lung environment and particularly with resident alveolar macrophages (AMs), remains limited. This knowledge gap limits our understanding of what drives protection or disease during MAB infection. To fill these important gaps, we leveraged a new *ex vivo* model of murine AMs known as fetal liver-derived alveolar-like macrophages (FLAMs). RNAseq analysis with a laboratory MAB strain comparing infection of bone marrow-derived macrophages (BMDMs) to FLAMs found that while BMDMs exhibit a strong pro-inflammatory phenotype, characterized by high TNF and chemokine secretion, they did not induce IL1α. FLAMs were hypoinflammatory except for the high expression of IL1α. *We hypothesize that IL1α has unique regulatory mechanisms and functional importance in AMs/FLAMs during MAB infection.* To test this hypothesis, we are dissecting the steps of IL1α regulation using a multi-pronged approach. First, we characterized host responses in AMs using 30 MAB clinical strains, revealing varied IL1α secretion despite most strains upregulating *Il1a* mRNA. Next, to understand how IL1α is regulated, we conducted a genome-wide forward genetic screen for the production of IL1α following TLR2 activation. Our results uncovered that core innate signaling pathways, AM-specific regulators, and post-translational modifiers were required for high ILα production. Ongoing validation studies are dissecting how these regulators modulate responses to distinct MAB clinical isolates. Finally, we are examining the role of IL1α and IL1 signaling in the initial host response in the lungs. Our preliminary data suggest that mice infected with clinical isolates result in major differences in the host response, and that IL1R signaling may be required to survive pulmonary MAB infection with a subset of strains. These data suggest strain-specific activation of innate immunity with differential IL1α as a distinguishing feature. These studies provide insight into how AMs contribute to protection or disease, uncovering potential therapeutic targets to combat MAB infection. More broadly, these studies will help define key regulatory mechanisms of inflammation in AMs that can be examined in the context of a wide range of pulmonary infections and diseases.

THE IMPACT OF THE TYPE VII SECRETION SYSTEM ON HOST RESPONSES TO GROUP B *STREPTOCOCCUS* IN THE FEMALE GENITAL TRACT

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Group B *Streptococcus* (GBS) is Gram-positive pathogen that asymptomatically colonizes the female genital tract (FGT) but can contribute to adverse pregnancy outcomes including pre-term birth, chorioamnionitis, and neonatal infection including pneumonia, bacteremia, and meningitis. GBS-induced cytokine responses have been well-studied during both systemic infection as well as during vaginal colonization and ascending infection. However, given its persistence within the vaginal tract, GBS has likely evolved mechanisms to evade clearance by inflammatory host responses. Despite this, the cellular immune response to colonizing GBS as well as the bacterial factors modulating host responses in this niche have not been well characterized. Type VIIb secretion systems (T7SSb) are encoded by Bacillota and secrete immunogenic effector proteins with functions in virulence, toxicity, interbacterial killing, and modulation of host responses. We previously identified a role for the GBS T7SS in pathogenesis during meningitis and, recently, demonstrated that T7SS also promotes persistence in FGT tissues; yet, the mechanisms by which the T7SS promotes GBS colonization remained unclear. Herein, we investigate a role for the GBS T7SS in subversion of FGT immune responses and characterize cellular responses to GBS during vaginal colonization and ascending infection by flow cytometry.

To investigate if inflammatory differences might drive differential clearance of parental and ΔT7SS GBS-colonized mice, we first assessed cytokine levels within colonized FGT tissue homogenates. Our laboratory has previously shown that IL-17 is associated with GBS clearance from the FGT. Interestingly, we observed increased IL-17 protein levels in the vaginal tissue of ΔT7SS mutant-infected mice (most of which had cleared) compared to parental GBS-infected mice (most of which remained colonized), indicating that the GBS T7SS may thwart IL-17 responses to promote vaginal persistence and ascending infection. This phenotype was also observed for IL-23, an upstream cytokine known to induce IL-17 production. To determine if this differential cytokine abundance is indicative of an altered immune response, we next developed innate and adaptive flow cytometric panels to assess cellular immune responses to persistently colonizing GBS. Consistent with the hypothesized immune-evasive nature of GBS in this niche, GBS colonization did not elicit a marked increase in CD45+ immune cells in FGT tissues compared to naïve animals over time. Despite this lack of dramatic response, we did observe significantly increased GBS burdens in FGT tissues in the absence of certain immune populations within the IL-17 axis, such as FGT-resident IL-17-producing γδ T cells (KO mice) and neutrophils (anti-Ly6G depletion). These data suggest that IL-17 responses in the FGT may control GBS colonization and our ongoing work seeks to determine if the T7SS subverts this protective host response, resulting in T7SS-mediated GBS FGT persistence.

REGULATORY ROLE OF THE HU PROTEIN IN *FRANCISELLA TULARENSIS* VIRULENCE

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Francisella tularensis, the causative agent of tularemia, is classified as a highly virulent biological threat due to its potential for aerosolization, which can lead to severe and often fatal pneumonic tularemia. This pathogen exhibits an exceptional ability to persist within phagocytic cells by evading the host immune response. Following internalization, *F. tularensis* escapes from the phagosome into the cytoplasm, where it undergoes extensive replication, ultimately inducing apoptosis in host cells. The molecular mechanisms underlying phagosomal escape and intracellular proliferation are largely attributed to proteins associated with an atypical type VI secretion system (T6SS), which is encoded by the *Francisella* pathogenicity island (FPI). However, in addition to FPI-encoded factors, numerous other proteins contribute to the virulence of *F. tularensis*, highlighting the complexity of its pathogenic strategy. Among these, the histone-like **HU protein** plays a pivotal role. The *Francisella* HU protein is involved in DNA structuring and regulation of gene expression, including virulence-associated genes. It has been shown to contribute to chromosomal organization and may influence the expression of FPI components as well as other genes important for intracellular survival and stress resistance, further underlining its significance in the pathogen's multifaceted approach to host manipulation.

CHARACTERIZING A NOVEL PAIR OF REDUNDANT *LEGIONELLA* VIRULENCE FACTORS

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Legionella pneumophila is a facultative intracellular pathogen that, when inhaled by humans, can infect and replicate within alveolar macrophages leading to a potentially fatal form of pneumonia called Legionnaires' disease. Once inside a macrophage, *L. pneumophila* translocates over 300 protein effectors into the host cell that establish the *Legionella*-containing vacuole and promote bacterial replication. Due to a high degree of redundancy between effectors, deletion of a single effector-encoding gene rarely produces a phenotype, making it difficult to determine their function. Using a multiplex CRISPR interference screen, we have identified a novel pair of transmembrane effector-encoding genes, *mieA* and *mieB*, that is necessary for efficient *L. pneumophila* growth in macrophages. Although these effectors share little sequence identify, their predicted protein structures are strikingly similar. Only upon deleting both genes is intracellular growth severely impacted, and this defect can be rescued by reintroducing either gene individually into the double deletion mutant, supporting the hypothesis that they are functionally redundant. Based on ongoing molecular and cell biology studies, we propose a transport function for this uncharacterized pair of *L. pneumophila* effectors.

BIOCHEMICAL CHARACTERIZATION OF NEUTRALIZING ANTIBODIES AGAINST *S. AUREUS* CYTOLYTIC PORE-FORMING TOXINS

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Staphylococcus aureus is a commensal bacterium that resides on the skin and soft tissue. Over one-third of people can be affected without causing harm. However, in immunocompromised individuals and surgical patients, a multitude of virulence factors drive infection. Methicillin-resistant *S. aureus* (MRSA) infections remain prevalent in community and healthcare settings, posing a global health issue that is challenged by adaptive antibiotic resistance and limited treatment options. Thus, attention has shifted toward targeting specific virulence factors that facilitate infection and immune evasion. Among these virulence factors, cytolytic pore-forming toxins (PFTs) play an important role in pathogenesis and disease progression. The secretion of PFT monomers by *S. aureus* enables recognition and interaction with membrane-bound host factors that are differentially expressed in erythrocytes and leukocytes. Upon binding to the putative host receptor, monomeric PFTs undergo a conformational change that shifts the rim domain from a solvent-protected state, as defined by the cap and stem domains, toward a solvent-exposed state, allowing for interaction with the host cell membrane. Membrane insertion and subsequent oligomerization result in a β-barrel that causes osmotic and ion gradient imbalances, inflammation, and activation of cell stress pathways.

Preliminary binding studies of a patient-derived monoclonal antibody (mAb) reveal a broad interaction profile that includes binding to alpha hemolysin (Hla), gamma hemolysin subunits, and leukocidin subunits. These observations suggest a broadly-neutralizing mode of action that hinders successful pore formation and suppresses disease phenotypes. However, it is unclear at which step of pore formation this interaction occurs and how binding may modulate pore formation mechanisms. Cryogenic-electron microscopy (cryoEM) and analysis reveal the conserved binding of the fragment antigen-binding region (Fab) to the rim domains of Hla, leukocidin F (LukF), leukocidin D (LukD), and gamma hemolysin subunit B (HlgB). We hypothesize that the mAb binds to PFT rim domains to modulate the conformational change of the monomer and downstream pore formation. The broadly neutralizing mode of action provides an effective prophylactic therapy when combined with other treatments. Ongoing work will identify the residues essential for mAb interaction with PFTs utilized in *S. aureus* infection. Together, these studies provide insights into the neutralization mechanism that disrupts pore formation.

SCREENING, CHARACTERIZATION AND IDENTIFICATION OF ANTI-ALPHA VIRUS COMPOUNDS

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Alphaviruses are a genus of arthropod-borne viruses (arboviruses) that can lead to two primary clinical manifestations in humans: arthritogenic condition, characterized by fever, rash, and prolonged polyarthritis (e.g., Chikungunya virus, Sindbis virus), and encephalitic conditions, marked by severe neurological complications (e.g., Venezuelan equine encephalitis virus). The emergence and re-emergence of alphaviruses pose significant global public health threats due to their potential for rapid geographical spread and lack of approved antiviral therapies. Despite their clinical significance, no therapeutic and prophylaxis are currently approved for human use against the pathogen, thus underscoring the need for effective antiviral therapeutics. In this context, the present study focuses on the screening and identification of potential anti-alphaviral compounds through a combination of in silico, in vitro, and cell-based assays. Comprehensive screening of a library of chemically synthesized compounds, followed by structure-activity relationship (SAR)-guided optimization, led to the identification of two lead candidates. The compounds demonstrated effectiveness against old world alpha viruses at nano molar concentrations with negligible cytotoxicity profiles in-vitro studies. Preliminary data suggest that these compounds interfere with the viral replication cycle, although the precise molecular targets are not yet fully defined, ongoing mechanistic studies are underway to pinpoint the specific interactions with viral nonstructural proteins. The findings will contribute to the growing body of research aimed at developing broad-spectrum antivirals and highlight promising candidates for further preclinical development against alphaviral infections.

DECODING THE ROLE OF C5A PEPTIDASE IN *STREPTOCOCCUS AGALACTIAE* VIRULENCE DURING COLONIZATION OF THE FEMALE REPRODUCTIVE SYSTEM

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Streptococcus agalactiae (Group B Strep, GBS) infections in neonates are often fatal and are strongly associated with maternal GBS vaginal colonization. The use of preventive intrapartum antibiotics, while effective against early onset disease, have their own pitfalls and have no effect on late onset neonatal GBS diseases. C5a peptidase (ScpB) is a conserved protein in all GBS strains and consist of two domains, the LPxTG motif and catalytic sites. *scpB* was knockdown using the CRISPRi-cas9 GBS system. Results highlight the important role of ScpB in evading the innate immune response during vaginal colonization primarily by impairing neutrophil recruitment and macrophage mediated killing. *In vivo* vaginal colonization corroborates the adhesive and invasive importance of ScpB that was identified *in vitro* using vaginal epithelial cells. Multiplex immunofluorescent imaging of colonized mouse vaginal tract and cervix indicate the direct engulfment of GBS by neutrophils at the epithelia as well as the activation of some adaptive immune response. Intramuscular and Intravaginal murine vaccination with purified recombinant ScpB protein stimulated a strong IgG response but not IgA, suggesting that these routes did not provide adequate immune protection from GBS infection. With this, nasopharyngeal inoculation is being conducted while simultaneously exploring a more innovative approach of creating, that is, GBS nanovaccine. The extracellular membrane vesicles of GBS are purified by filtration and ultracentrifugation then combined with a STING core to form the nanovaccine. We anticipate a more robust immune response and extended protection because STING is a strong driver of stimulating the switch from an IgG to IgA response.

PROTEASE CLEAVAGE POTENTIATES *STREPTOCOCCUS PNEUMONIAE* PNEUMOLYSIN ACTIVITY

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Streptococcus pneumoniae (pneumococcus) colonizes the nasopharynx of 25-50% of the population and is the leading cause of community acquired pneumonia and acute bacterial otitis, affecting millions of people worldwide. One of the primary virulence factors expressed by pneumococcus is the pore-forming toxin pneumolysin (PLY). PLY is multifunctional with cytolytic, complement-activating, and immunomodulatory functions. While we know much about this toxin, many questions remain regarding factors regulating its activity. Like other cholesterol-dependent cytolysins (CDCs), PLY consists of four domains. It is well established that the toxin initially oligomerizes to form a pre-pore in host cell membranes prior to undergoing a conformational change resulting in membrane insertion and complete pore formation. However, it is unknown if external factors such as protease cleavage can affect this process. Our hypothesis is that the cellular effects of PLY, both cytotoxic and inflammatory, are enhanced by protease cleavage and impact pathogenesis. Here we demonstrate that protease cleavage indeed enhances PLY toxicity. In the process of analyzing the adhesion of pneumococcus to A549 human lung epithelial cells, we identified that at certain multiplicities of infection the cells began to bleb following application of trypsin. The addition of EDTA greatly enhanced this effect, likely due to a key role for calcium in recovery from CDC-induced membrane damage. The appearance of the cells clearly indicated that the cell membrane was being compromised. We identified that PLY was responsible for the loss of membrane integrity by exposing A549 cells to an isogenic mutant lacking the ply gene (ΔPLY). Blebbing was not observed following exposure to trypsin. Additionally, we were able to reproduce this effect by exposure of A549 cells to recombinant PLY (rPLY) and subsequent trypsin treatment. The hyper-lytic effect occurred within 5 min following trypsin exposure. However, exposure of cells to trypsin without prior incubation with bacteria or rPLY never induced cell lysis, even after 30 minutes of incubation (data not shown), indicating that the effect was due to an interaction between PLY and trypsin. Incubation of rPLY with trypsin prior to exposure to A549 cells led to no cell lysis even at cytotoxic concentrations, indicating the toxin had been inactivated by cleavage. These results suggest that PLY must bind to cellular membranes prior to trypsin treatment for the hyper-lytic effect to occur. Additionally, we investigated the ability of human airway trypsin and neutrophil proteases to enhance PLY-mediated lysis. Given that individual domains of PLY are known to elicit responses within immune cells, including activation of Th17 T-cells and apoptosis in macrophages, cleavage of the toxin could liberate fragments with enhanced immuno-modulatory effects. This work has potential to change our understanding of pneumococcal pathogenesis and will add an additional target for future therapeutics for invasive disease.

IMPAIRED DNA REPAIR PROTEIN ENHANCES BIOFILM DEFENSES AGAINST ANTIBIOTICS IN *ACINETOBACTER BAUMANNI*

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Minocycline (MIN), a broad-spectrum tetracycline-class antibiotic, is a vital therapeutic option for infections caused by multidrug-resistant *Acinetobacter baumannii* (Ab). Within the human host, Ab rapidly establishes biofilms on respiratory epithelium lessening innate immune recognition and hindering antibiotic penetration. Despite its clinical value, resistance to MIN is increasingly reported in Ab isolates, threatening its therapeutic utility. Although efflux pumps such as TetB are well-characterized in other bacteria and commonly linked to tetracycline resistance, they do not fully account for MIN resistance observed in Ab isolates. This suggests the involvement of additional, previously unrecognized mechanisms of resistance. Through machine-learning-based analysis of over 1,440 Ab clinical genomes, we identified significant correlations between MIN resistance and mutations in ruvB, a gene encoding a DNA helicase in the RuvABC Holliday-junction resolvase complex. Mutations in ruvB have not previously been associated with MIN resistance.

We utilized two Ab strains: the multidrug-resistant AB5075 and the antibiotic-susceptible ATCC 19606—to examine how ruvB mutations impact MIN resistance. In both, disruption of ruvB led to a substantial increase in MIN resistance and a dramatic enhancement in biofilm formation. Biofilms from ruvB disruption contained markedly higher concentrations of extracellular DNA (eDNA). During DNA repair by the RuvABC system, RuvA first binds Holliday junctions and then recruits RuvB, which drives branch migration of the DNA strands. We therefore hypothesize that loss of RuvB allows unbound RuvA to stabilize eDNA. Restoring ruvB reversed these effects. RuvB mutants also showed increased resistance to other antibiotics, suggesting freeing of RuvA may confer a broad, biofilm-driven mechanism of resistance in Ab.

In conclusion, our findings suggest that ruvB mutations are key drivers of biofilm-mediated MIN resistance in Ab through RuvA stabilization of eDNA. This previously unrecognized mechanism underscores the intersection of biofilm physiology, immune evasion, and antibiotic resistance, highlighting RuvB as a potential dual-function target for disrupting Ab pathogenesis and restoring antimicrobial efficacy. Elucidating how RuvB dysfunction shapes host–pathogen interactions may guide development of host-directed strategies to enhance immune clearance of chronic Ab infections.

DEFORMED WING VIRUS REPROGRAMS HOST BRAIN METABOLISM TO PROMOTE JUVENILE HORMONE BIOSYNTHESIS IN HONEYBEES (*APIS MELLIFERA*)

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Honeybee (*Apis mellifera*) populations have been declining in recent years, partly due to viral pathogens such as Deformed Wing Virus (DWV). DWV is highly prevalent and causes both morphological, physiological and behavioral impairments, including deformed wings, labor early division and memory loss, eventually contributes to colony loss. Although juvenile hormone (JH) and nutritional status are both known to influences the division of labors, the underlying physiological mechanisms linking DWV-induced metabolic changes to hormonal regulation has yet be exposed. Furthermore, no study has directly connected viral infection, energy metabolism, and JH-mediated behavior in honeybees.

In this study, we focused on metabolic alterations in the honeybee brain to investigate the interaction between DWV infection and host physiology. Using DNS assays and ELISA, we found significant reductions in glucose, ATP, and NADH levels in DWV-infected bees. Furthermore, the expression of genes involved in juvenile hormone (JH) biosynthesis was altered. We further demonstrated that DWV infection enhances the pentose phosphate pathway (PPP), an alternative metabolic route, as evidenced by RT-qPCR and enzyme activity assays. One of its key products, nicotinamide adenine dinucleotide phosphate (NADPH), supports the activity of methyl farnesoate epoxidase (MFE), which in turn promotes JH production. The metabolic regulator, adenosine, was supplemented to reverse the altered metabolic state, resulting in recovering of glucose, ATP levels and decreasing activity of enzyme in PPP and MFE gene expression. The present study discovered a novel DWV parthenogenesis on honeybee's hormone regulation. A potential supplement was also suggested for future utilization on apiculture. Our findings reveal how virus reprograms host metabolism and hormonal signaling, and raise the question of whether these metabolic changes represent a host-derived defense mechanism or a strategy exploited by the pathogen.

INTRA- AND INTER-INDIVIDUAL STRAIN TRACKING OF COAGULASE-NEGATIVE *STAPHYLOCOCCI* IN THE PRETERM GUT MICROBIOME LINKED TO LATE-ONSET SEPSIS

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BACKGROUND: Late-onset sepsis (LOS), or bloodstream infections (BSIs) ≥72 hours after birth, is a leading cause of morbidity and mortality in preterm infants. Coagulase-negative *Staphylococci* (CoNS) are the most frequently identified organisms in blood cultures from such infants. CoNS are presumed to gain access from the skin or by contamination of indwelling devices, but are prevalent and dominant members of the preterm gut microbiota. Here, we test the hypothesis that the gut (1) can be a habitat for LOS-causing CoNS, and (2) is within a chain of transmission and persistence within the neonatal intensive care unit (NICU).
METHODS: We identified 51 cases where CoNS were isolated from blood ≥72 hours after birth in a cohort of 977 preterm infants (<37 weeks gestation) hospitalized across 3 Midwestern U.S. NICUs from 2009-2013. From this primary cohort, we whole genome-sequenced 49 CoNS isolates from blood and shotgun-sequenced 34 stools collected ≤5 days before LOS. Isolate genomes were assembled *de novo*, and pairwise genetic distances were measured by variant calling and population average nucleotide identity (popANI) using inStrain. Stool metagenomic reads from CoNS BSI cases, non-CoNS BSI cases, and negative controls (no BSIs) were aligned to CoNS BSI isolate assemblies, followed by popANI calculation. To assess longitudinal NICU presence of BSI-causing CoNS, isolates and stools from the primary cohort were compared to those from a secondary cohort consisting of 8 CoNS+ LOS cases from one of the same NICUs in 2022.
RESULTS: In the primary cohort, the most common species isolated from CoNS BSI cases were *Staphylococcus epidermidis* (41%) and *Staphylococcus capitis* (31%). Comparative strain analysis identified 7 multi-strain clusters with popANI ≥99.99%. For 5/32 (16%) cases with paired blood & pre-BSI stools analyzed, the BSI isolate was identified in the gut before LOS onset. Out of 162,680 isolate-metagenome comparisons, we detected 45 inter-individual strain-sharing events of CoNS BSI isolates within CoNS BSI case metagenomes and 813 events between non-CoNS BSI and non-BSI control metagenomes. In the secondary cohort, the BSI strain was detected in the gut before LOS onset in 1/8 cases. Cross-cohort analysis identified 462 inter-individual strain-sharing events between primary cohort gut metagenomes and secondary cohort BSI isolates, suggesting long-term environmental persistence.
CONCLUSIONS: In a subset of infants, the CoNS BSI strain resides in the gut prior to dissemination, and hospitalized infants harbor CoNS strains with BSI-causing potential, thereby identifying a modifiable risk factor for CoNS BSIs.

A PROTECTIVE ROLE FOR THE LECTIN CD169/SIGLEC-1 DURING SARS-COV-2 INFECTION

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The sialic acid binding lectin Siglec-1/CD169 plays a critical role in innate immune surveillance by capturing and presenting viral particles to immune cells, making it a key mediator in antiviral defense. Analogous to sentinel macrophages located at tissue-lymph/blood interfaces, subpopulations of murine pulmonary macrophages also express CD169 that bind both host and pathogen-associated sialosides. Ex-vivo studies indicate that the CD169 expression promotes SARS-CoV-2 *trans*-infection and exacerbates macrophage inflammatory responses contributing to disease pathology. However, the *in vivo* consequences of CD169-mediated activities during respiratory viral assault remain unclear. We find that, in contrast to $B6_{WT}$ mice, infection with the model respiratory pathogen, mouse-adapted SARS-CoV-2 (MA10) is lethal in $CD169^{-/-}$ mice. CD169 expression enabled the capture of incoming viruses by pulmonary macrophages, reduced its spread to alveolar epithelial cells 2 (AT2), promoted early induction of anti-inflammatory cytokine IL-10, curtailed the inflammatory phase and promoted virus clearance. Conversely, CD169 ablation impaired virus sequestration and enhanced spread to AT2 cells and drove prolonged inflammation. Inhibiting the inflammasome pathway with NLRP3 and Caspase-1/4 inhibitors prevented virus-induced mortality in $CD169^{-/-}$ mice. Thus, contrary to a predicted detrimental role, CD169 was a protective host factor that facilitated balanced immune response to resolve SARS-CoV-2 infection with minimum host pathology.

A SYNTHETIC BOTTOM-UP PLATFORM REVEALS DISTINCT ROLES OF *SHIGELLA* OSPC1 AND OSPC3 EFFECTORS IN MODULATING HOST CELL DEATH PATHWAYS

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Shigella spp., the etiologic agents of bacillary dysentery, rely on their type III secretion system (T3SS) to inject ~30 effector proteins into host intestinal epithelial cells (IECs), facilitating intracellular survival and replication. The functional redundancy among effectors has historically hindered dissection of their individual contributions to pathogenesis. Here, we present mT3Sf, a synthetic bottom-up platform designed to evaluate the role of individual *Shigella* effectors in infection. This platform comprises a virulence plasmid cured *S. flexneri* strain re-engineered to chromosomally encode the T3SS apparatus and express effectors individually from inducible plasmids. Using mT3Sf, we found that infection of IFNγ-primed HeLa cells in the absence of any effectors (mT3Sf_EV) triggers a biphasic programmed cell death response: early pyroptosis dependent on caspase-4 and Gasdermin D, followed by apoptosis at later time points, as evidenced by caspase-3/7 activation and phosphatidylserine externalization. In GSDMD$^{-/-}$ and caspase-4$^{-/-}$ HeLa cells, pyroptosis was abolished, but apoptosis-like death emerged with delayed kinetics, suggesting the sequential engagement of distinct death pathways. Using this system, we evaluated the roles of effectors OspC1 and OspC3 by comparing the fate of cells infected with mT3Sf_OspC1, mT3Sf_OspC3 and mT3Sf_EV. The presence of OspC3 effectively blocked early pyroptotic death of infected wild-type cells, consistent with its known ability to ADP-riboxinate and inactivate caspase-4. Surprisingly, the presence of OspC3 in mT3Sf infected GSDMD$^{-/-}$ cells partially suppressed death, indicating a previously unrecognized anti-apoptotic function. In contrast, the presence of OspC1, previously implicated in apoptosis inhibition, resulted in a markedly stronger suppression of caspase-3/7 activity, phosphatidylserine exposure and death of infected GSDMD$^{-/-}$ HeLa, but had limited effect of death of WT cells. The presence of both effectors reduced cleavage of apoptotic initiator caspases-8 and -9 and executioner caspases-3 and -7. In follow-up co-transfection of HEK293T, we observed that both effectors can ADP-riboxinate caspases-3, -7, -8, and -9, in a manner dependent on their catalytic activity. Collectively, these observations demonstrate that mT3Sf is a powerful gain-of-function screening platform for dissecting the roles of individual *Shigella* effectors. Using this platform, we have established that *Shigella*-induced cell death likely involves a temporal switch from pyroptosis to apoptosis and that effectors OspC1 and OspC3 can both suppress apoptotic pathways, uncovering a dual role for OspC3 in blocking both pyroptotic and apoptotic pathways. These findings underscore the versatility of *Shigella* T3SS effectors in modulating host cell fate and highlight the utility of bottom-up approaches to parse complex effector networks.

EVOLUTION AND PATHOADAPTATION OF *PSEUDOMONAS AERUGINOSA* THROUGH THE GUT-LUNG AXIS IN CYSTIC FIBROSIS

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People with cystic fibrosis (pwCF) face significant challenges with dyslipidemia linked to chronic lung complications. Despite advances with highly effective modulator therapy (HEMT), pathogens like Pseudomonas aeruginosa (Pa) persist, as does dyslipidemia. Pa isolates collected from pwCF show that new variants after HEMT have significantly increased mutations in lipid metabolism genes, suggesting that Pa is adapting to an environment with altered lipid profiles. The "gut-lung axis" suggests that gut microbiota, immune regulation and nutrients from the gut can alter lung health, with GI dysbiosis being a predictor of pulmonary exacerbations in pwCF. Dietary fatty acids are converted into chylomicrons in the small intestine and enter the blood via lymph before reaching the heart and lungs. To determine if dietary lipids are conveyed to the airways, we gavaged mice with 3H-oleate and tracked dietary lipid deposition. We observed significant dietary lipid accumulation in the airways (bronchoalveolar lavage fluid - BALF), which was confirmed with immunofluorescence imaging in CF bronchial epithelial cell cultures (CFBEs). We next used experimental evolution to define how Pa evolves with this new nutrient source. We passage Pa (lab strain MPAO1) on CFBEs basolaterally exposed to gut-derived chylomicrons and monitored the evolution of Pa populations over time. Whole-genome sequencing revealed mutations in fatty acid degradation genes (fadA, fadD, fadE, and fadJ) in Pa populations that had been passaged over chylomicron-treated CFBEs and clinical isolates collected from pwCF post-HEMT. Further analysis revealed that these mutations confer an adaptive advantage to Pa under specific nutritional conditions. Representative transposon mutant Pa strains with deletions in fadE (tnFadE) exhibit increased resistance to tobramycin in biofilm assays and increased survival when primed with long-chain fatty acids (LCFAs) in macrophage killing assays. Competitive fitness assessments of Pa tnFadE revealed dramatic shifts in a model CF respiratory microbiome, whereby Pa tnFadE abundance increased relative to WT, while other microbiota depleted in its presence. These findings provide an explanation for Pa persistence in the post-therapy CF lung and propose a novel paradigm in the gut-lung axis that explores how gut-derived lipids shape the nutritional environment of the lung and drive adaptation of pathogens that cause chronic infections.

INHIBITION OF RND-MEDIATED EFFLUX ATTENUATES ANTIBIOTIC RESISTANCE AND VIRULENCE IN *KLEBSIELLA PNEUMONIAE*

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Klebsiella pneumoniae is a major human pathogen responsible for causing both hospital and community-acquired infections. Hypervirulent strains of *K. pneumoniae* (hvKp) pose a significant threat to public health due to their ability to cause severe invasive infections in otherwise healthy individuals. While antimicrobial resistance is a major concern for Kp infection biology, the production of virulence factors such as capsule, biofilm formation, and iron acquisition systems play significant roles in hvKp pathogenesis. In this work, we investigated the contribution of resistance-nodulation-division (RND)-family efflux systems to antimicrobial resistance and virulence in hvKp strain KPPR1 using the RND-specific inhibitor phenyl-arginine-β-naphthylamide (PAβN). We found that PAβN treatment rendered KPPR1 hypersensitive to multiple antibiotics. Further, PAβN significantly reduced capsule production as documented by decreased uronic acid levels and decreased expression of capsule biosynthesis genes. Hypermucoviscosity, a characteristic phenotype of virulent strains of hvKp, was significantly reduced in PAβN treated cells. Additionally, PAβN inhibited biofilm formation, suggesting RND-mediated efflux is necessary for the production of robust biofilm in KPPR1. Interestingly, treatment with PAβN resulted in impaired growth under iron-limited conditions, suggesting a role for RND-mediated efflux in hvKp iron metabolism. PAβN-treated KPPR1 showed reduced adherence to cultured intestinal enterocytes and attenuated virulence in the *Galleria mellonella* infection model compared to untreated controls. Collectively, these results demonstrated that RND-mediated efflux is critical for both antimicrobial resistance and virulence in hvKp strain KPPR1. Our findings establish RND efflux inhibitors as potential dual-target therapeutics that can simultaneously combat antibiotic resistance and attenuate virulence in hvKp.

LISTERIA MONOCYTOGENES PEPTIDOGLYCAN ENDOPEPTIDASES PROMOTE PATHOGENESIS AND MAY CONTRIBUTE TO THE DEVELOPMENT OF LONG-TERM IMMUNITY.

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The bacterial cell wall provides protection against external stressors, yet how cell wall turnover impacts bacterial fitness and host innate immunity is unclear. It is predicted that the autolytic enzymes responsible for cell wall turnover in the model Gram-positive facultative intracellular pathogen *Listeria monocytogenes* release *N*-acetylglucosaminyl-*N*-acetylmuramyl dipeptide (GMDP) that is recognized by the host innate immunity receptor protein NOD2. GMDP is an analog of MDP (muramyl dipeptide), a potent immune adjuvant recognized by NOD2 known as the minimally active component of Freund's complete adjuvant. This prompted us to examine the role of each of the annotated endopeptidases, *p60*, *p45*, and *lmo0394*, that are predicted to cleave the peptide stem of peptidoglycan and release GMDP. P60 is highly secreted by *L. monocytogenes* and was necessary for efficient bacterial septation, as mutants lacking *p60* grew as chains and were 50-fold less virulent in mice. P45, previously thought to be essential, supported *in vivo* infection as *p45* mutants were 200-fold less virulent in mice. However, in contrast to *p60* mutants, *p45* mutants did not suffer from cell wall chaining defects and behaved like wild type in all *in vitro* virulence assays. Surprisingly, a Δ*p45*Δ*p60* double mutant rescued the cell chaining defect of a Δ*p60* single mutant but had an additive virulence defect. No defects were observed in single mutants lacking *lmo0394*. Using a triple mutant deficient for each of the three endopeptidases, we are currently assessing the role of *L. monocytogenes* endopeptidase function in pathogenesis and their role in GMDP release and NOD2 activation, CD8+ T cell production, and adaptive immunity. A distinguishing feature of *L. monocytogenes* pathogenesis is its ability to generate a robust CD8+ T cell response and long-term adaptive immunity and has been used as a model pathogen to interrogate links between innate and adaptive immunity. We hypothesize that *L. monocytogenes* endopeptidases support pathogen fitness but concomitantly release peptidoglycan fragments triggering a NOD2 dependent innate immune response necessary for the development of long-term immunity. Broadly, this work expands upon our knowledge of bacterial cell wall turnover in the host cell environment and addresses whether there is a yet undiscovered link between innate and adaptive immunity driven by NOD2 activation in response to bacterial pathogens.

UNDECAPRENYL PYROPHOSPHATE PHOSPHATASE (UPPP) IS A PIVOTAL ELEMENT IN *SALMONELLA* INTRAMACROPHAGE SURVIVAL

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Salmonella, an enteric pathogen, encounters several stresses while infecting the host, such as oxidative, nitrosative stress, acidic pH, nutrient starvation, metal ions, and host antimicrobials. The peptidoglycan layer plays an essential role in countering these by acting as a barrier to the external environment. Multiple genes work together synergistically to successfully synthesise peptidoglycan in *Salmonella*. Amongst all, the Undecaprenyl pyrophosphate phosphatase (UppP) catalyses the dephosphorylation of undecaprenyl pyrophosphate to undecaprenyl phosphate, which serves as the lipid carrier for peptidoglycan synthesis. Previous studies in *Streptococcus pneumoniae* and *Helicobacter pylori* have highlighted the important role of UppP in successful pathogenesis and colonisation, respectively, in the mouse model. However, the role of UppP in *Salmonella* virulence and pathogenesis is understudied. Generation of the *uppP* mutant in *Salmonella* Typhimurium (STM) revealed that UppP is essential for maintaining bacterial length and Young's modulus in *Salmonella*. Further, our results show that UppP supports STM survival and proliferation inside RAW264.7 macrophages and is crucial for systemic dissemination and survival within the C57BL/6 mouse infection model. The reduced intracellular proliferation of the mutant in macrophages can be attributed to its enhanced susceptibility to nitrosative stress, as observed in our study. Moreover, our investigation also revealed that STM *ΔuppP*-infected RAW264.7 macrophages exhibited elevated levels of reactive nitrogen species (RNS), which is primarily due to the induction of inducible nitric oxide synthase (iNOS) through NOD2 (Nucleotide-binding oligomerisation domain containing protein 2) receptor signalling. Furthermore, abrogation of either iNOS or NOD signalling rescues the attenuated proliferation and survival of the *uppP* mutant within RAW 264.7 macrophages. Similarly, in iNOS-deficient mice, the *uppP* mutant and wild-type strain had a similar organ burden. In summary, our study delineates the moonlighting function of UppP in withstanding nitrosative stress within macrophages and aiding in *Salmonella* pathogenesis.

CHARACTERIZING INTERACTIONS BETWEEN RSV AND THE ACTIN CYTOSKELETON

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Human respiratory syncytial virus (RSV) infects the lower respiratory tract, with infants, young children under 2, and adults with compromised immune systems particularly susceptible. RSV infection causes a significant number of hospitalizations and is a serious public health threat.
The actin cytoskeleton is a highly dynamic network that is important for maintaining cellular architecture and signaling processes. It can reorganize very rapidly in response to changing conditions in the cell and depends on many actin-related and -binding proteins. Given its importance for cellular function, viruses have developed strategies to hijack the actin cytoskeleton to facilitate nucleocapsid assembly, budding, and egress.
Here we describe our recent efforts to identify and characterize specific interactions between RSV proteins and the actin cytoskeleton. We use a combination of biochemical and cell biological assays to show that RSV M directly impacts actin polymerization. Specifically, we show that RSV M impacts actin processes that are dependent upon the Arp2/3 complex nucleator. Furthermore, we find that RSV M is localized with actin near viral inclusion bodies where viral replication occurs and at membrane protrusions. Our work collectively points to a new potential therapeutic target for RSV infections.

CHANGES IN HOST DEUBIQUITINATING ENZYME ACTIVITY DURING *FRANCISELLA TULARENSIS* INFECTION OF HUMAN MACROPHAGES

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This study focused on investigating changes in the relative abundance and activity of host deubiquitinating enzymes (DUBs) during infection with the Gram-negative bacterium *Francisella tularensis*. Human macrophages differentiated from the THP-1 monocytic cell line were infected with *F. tularensis* subsp. *holarctica* FSC200, and samples were collected at an early infection time point (60 minutes). DUBs were analyzed both in whole cells and in extracellular vesicles released by infected cells.
Multiple methodologies were employed to assess DUB changes: (1) proteomic profiling via LC-MS/MS using enzymatically digested peptides with TMT isobaric labeling and label-free quantification (LFQ), complemented by Western blotting; and (2) gene expression analysis using real-time PCR. Bioinformatic analysis of LC-MS/MS data revealed no significant changes in the relative abundance of cellular DUBs, a result further supported by Western blot and RT-PCR.
To assess enzymatic activity, two activity-based probes — HA-Ub-PA and HA-Ub-VME — were used for enrichment of active DUBs, followed by LC-MS/MS and Western blot analysis. This approach revealed significant activity changes in three DUBs: USP10, UCH-L5, and USP25. Parallel LC-MS/MS analysis of extracellular vesicles from infected cells also detected alterations in these same DUBs, which were again confirmed by Western blotting.
Overall, this study highlights infection-induced changes in the activity, but not abundance, of specific DUBs, offering new insights into the potential roles of USP10, UCH-L5, and USP25 in the host response to *Francisella tularensis* infection.

FACTORS DRIVING DISTINCT PHASE VARIABLE COLONY MORPHOLOGIES IN *CLOSTRIDIOIDES DIFFICILE*

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Clostridioides difficile is a Gram-positive pathogen that causes a range of gastrointestinal (GI) diseases, usually following antibiotic use that depletes normal microbiota. While it has long been a leading cause of nosocomial infections, community acquired *C. difficile* cases are on the rise, and reports of disease recurrence are becoming more frequent.

C. difficile faces numerous environmental challenges in the GI tract, and one mechanism by which it overcomes them is through phase variation of numerous phenotypes. One such phenotype is colony morphology, where colonies are either smooth or rough. We previously found that the rough variant trends towards more severe disease than the smooth variant in a hamster model of acute infection. In addition, we identified specific growth conditions that promote one morphotype over the other, suggesting environmental pressures influence morphology. Further work in our lab established that the orientation of an invertible DNA element upstream of an operon encoding the CmrRST phosphorelay system mediates the phenotypic switch. In one orientation, *cmrRST* are highly expressed and the colonies are rough. In the other orientation, *cmrRST* are weakly expressed and the colonies are smooth. In addition to DNA inversion, numerous other factors regulate *cmrRST* expression—a hint that production of these regulatory proteins, and the phenotypes they influence, needs to be finely tuned.

CmrR and CmrT are DNA binding response regulators. Their regulons have considerable overlap, but they also appear to have distinct roles. One CmrRST-regulated gene encodes a small protein that interacts with the cell division protein MinD, implicating altered cell division processes in the development of rough colonies. However, follow-up studies on other CmrRT-regulated genes failed to identify genes required for either colony morphology. We therefore undertook suppressor and transposon mutagenesis screens using a Δ*cmrRT* mutant, which forms strictly smooth colonies, to identify mutations that confer a rough colony phenotype. To date, over 25 mutants have been isolated and sequenced. Approximately 60% have mutations that affect known or predicted cell division genes, including four independent mutations in *ftsZ*, four in *zapA*, and one in *minD*. The bulk of the remaining mutations are in genes with predicted roles in cell division and transcriptional regulation. *C. difficile* lacks some of the known components of the gram-positive cell divisome, and cell division is not well understood in this pathogen. We anticipate that our studies will not only determine how CmrRST controls colony morphology but also reveal previously unknown factors mediating cell division.

CANDIDA ALBICANS ENHANCES EPIDERMAL PROTEOLYTIC ACTIVITY AND BARRIER DISRUPTION THROUGH MMP-9 ACTIVATION

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Candida albicans is enriched on atopic dermatitis (AD) skin and positively correlates with disease severity. Two hallmarks of AD include an inflamed skin environment and heightened proteolytic activity in the epidermis, yet *C. albicans* contribution to these pathological features is understudied.

We hypothesized that *C. albicans* triggers endogenous keratinocyte (KC) proteolytic activity, amplifying inflammation and barrier disruption. Our results revealed that KC exhibited a higher level of matrix metalloprotease-9 (*MMP9*) mRNA transcripts (15.98±9.91-fold, $p<0.01$) post exposure to *C. albicans* [10^3 colony forming units]. Western blot analysis corroborated this, demonstrating elevated MMP-9 translation and maturation, as evidenced by increased pro- and active forms (4.13±2.03 & 4.98±3.11-fold, respectively, $p<0.05$). This MMP-9 induction was accompanied by enhanced total protease activity compared to unexposed KC (13.40±3.32-fold, $p<0.01$). Notably, this response was attenuated in co-cultures with a *C. albicans* mutant lacking secreted aspartyl protease 4, 5, and 6, showing a 2.96±0.24-fold reduction ($p<0.05$).

Pro-inflammatory cytokines are known to modulate host proteases. We found that IL-1β treatment (1 ng/ml) also promoted maturation of MMP-9 in KC (4.37±0.802-fold, $p=0.06$). To link this finding to *C. albicans*, we confirmed that it induces significant IL-1β secretion from KC (195.34±24.31 pg/ml, $p<0.0001$). This response required both fungal virulence factor candidalysin and hyphal formation, as neither alone was sufficient. To identify host signaling pathways in this response, we used CRISPR-Cas9 to knockout (KO) key adapter molecules from KC (MyD88, NLRP3). IL-1β release was markedly reduced in MyD88-KO KC upon *C. albicans* exposure (29.32±9.97-fold, $p<0.05$), but unaffected by NLRP3 deletion, pointing to a non-classical and potentially inflammasome-independent mechanism of IL-1β processing. Finally, we examined the functional impact of IL-1β on epidermal barrier via transepithelial electrical resistance (TEER). IL-1β significantly impaired barrier function at day 5 ($p<0.01$) & 6 ($p<0.05$) post-differentiation, suppressing TEER to a maximum of 44.03±12.31% of untreated controls. Of note, KO of either *MYD88* (completely) or *MMP9* (partially) rescued barrier function, implicating an unappreciated role of these proteins in barrier function during inflammation.

Our findings suggest that skin microbiome facilitates traits indicative of AD pathogenesis, including KC-mediated proteolysis and inflammation. Ongoing work will extend these results to 3D epidermal models and skin explants to better capture the cellular complexity of a stratified tissue.

ISOTOPE TRACING AND METABOLOMICS REVEAL METABOLITE DETERMINANTS OF *LEPTOSPIRA INTERROGANS* PHYSIOLOGY UNDER HOST-LIKE CONDITIONS.

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Leptospirosis is a global re-emerging zoonotic infectious disease which impacts over one million people annually. Caused by pathogenic *Leptospira* spp. such as *Leptospira interrogans*, little is known about the mechanisms of bacterial persistence and pathology, including how cellular-level metabolism controls host responses and bacterial pathogenesis. To assess bacterial metabolism under host-like conditions, we first profiled small molecule nutrients assimilated by *L. interrogans* from a complex host-like medium, supplemented human plasma-like medium (sHPLM), with high-resolution liquid chromatography mass spectrometry (LC/MS) measurements on media metabolome extracts. In sHPLM (*in vitro*), *L. interrogans* growth rate matched reported in-host doubling times (9-13h/doubling). Metabolomics profiling revealed 11 putative nutrients, including the amino acid glutamine, small carbon metabolites such as pyruvate, and fatty acids. Fatty acids are a well-known carbon source for *Leptospira* species. Pyruvate was reported to increase the growth rate of *L. interrogans*, and we observed that it was also secreted by infected human macrophage cultures. Further study of glutamine metabolism with time-resolved ^{15}N stable isotope tracing on intra-bacterial metabolome extracts from *L. interrogans* sHPLM cultures allowed us to develop a phenotype-based map of nitrogen metabolic flux, superseding previous genome-based maps. Surprisingly, this determined that glutamine assimilation rivals ammonium assimilation, previously considered the sole nitrogen source. Consistently, a GlnA inhibitor blocking ammonium incorporation has little effect on proliferation in the complex sHPLM; however, a prodrug with a close analog in clinical trials that blocks glutamine-utilizing metabolic reactions strongly inhibited *L. interrogans* proliferation in sHPLM cultures. We also found that physiological concentrations of glutamine increased biofilm formation, an important factor for persistence, and yielded a rapid and transient proliferative bloom in pathogenic *L. interrogans* when cultured in a standard unphysiological medium, but failed to affect the non-pathogenic strain, *Leptospira biflexa* Patoc. However, these two effects were unrelated to glutamine's role as a nitrogen source. This work demonstrates the control of specific metabolic pathways and nutrients over *L. interrogans* physiology in host-like environments as well as the potential for glutamine, a host-associated metabolite, to act as a signal to change pathogen behavior. Studies to better understand the effect of glutamine and the role of nitrogen sources and pyruvate during host infection are ongoing.

MICROBIAL COMMUNITY DYNAMICS AND THEIR IMPACT ON EXPERIMENTAL OUTCOMES IN MURINE MODELS

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Understanding the dynamics of microbiota assembly in mouse models is paramount given their widespread use and importance in biomedical research, particularly the study of host-microbe interactions. While previous studies have touched upon these dynamics, they often lacked the scope necessary to elucidate the complexities involved. In this study, we present a thorough characterization of colonization processes and microbial community assembly across three independent yet related murine systems: the early development of the neonatal gut, colonization of germ-free mice, and following antibiotic treatment.

Our investigation spans multiple dimensions, including mouse genetic strains, vendors, campus facilities, and replicate experiments over time, providing a robust framework for understanding microbiota dynamics in murine models. By profiling bacterial and viral communities across these diverse contexts, we uncover nuanced insights into the factors shaping microbiota composition and its downstream effects on experimental outcomes.

Of particular significance is our demonstration of how variation between facilities on campus can impact phenotypic variation, notably in one of the most used experimental models of colitis. This underscores the importance of considering microbiota dynamics in experimental design and highlights the need for strategies to mitigate variability and enhance reproducibility.

These findings are especially relevant for researchers studying host-microbe interactions, as murine models remain essential tools for probing the microbial contributions to immunity, inflammation, and disease. Our work not only serves as a crucial resource for researchers working with mouse models—particularly those studying microbiome-mediated traits—but also offers actionable recommendations to address the pressing issue of reproducibility in biomedical research. By providing a comprehensive understanding of microbiota dynamics in murine models, this work paves the way for more robust experimental design and interpretation.

IMPACT OF ORAL ANAEROBES ON *STAPHYLOCOCCUS AUREUS* IN A CYSTIC FIBROSIS MODEL SYSTEM

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Cystic fibrosis (CF) is a life-threatening genetic disease that affects over 100,000 people worldwide. CF disease results from mutation of a gene for a cellular ion channel protein, the cystic fibrosis transmembrane conductance regulator (CFTR). Mutations in CFTR cause a variety of intestinal and pulmonary symptoms in part because of improper mucus clearance from epithelial cell surfaces. In the lungs, this mucus accumulation leads to chronic polymicrobial infections, inflammation, and permanent tissue damage, making pulmonary complications the primary cause of morbidity and mortality in people with CF. Approximately 60% of these chronic, recalcitrant, polymicrobial CF lung infections involve the major pathogen *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA). These infections also contain transcriptionally active oral commensal bacterial genera, including *Prevotella*, *Rothia*, and *Veillonella*, at high relative abundances. In our recent analysis of 31 CF sputum transcriptomes, these oral anaerobes (OAs) were negatively correlated with *S. aureus*, indicating possible competitive, inhibitory, or antagonistic interactions between these microbes and *S. aureus*. However, the specific interactions that occur between *S. aureus* and OAs are not well characterized, especially in the context of CF lung infection. Therefore, we employed an *in vitro* CF system (SCFM2), cell-culturing, metabolomics, and transcriptomics to elucidate mechanisms of interaction between *S. aureus* and OAs. Our goal is to determine how these OAs modify the growth and physiology of *S. aureus* in the complex CF lung environment, ultimately improving our understanding of these difficult to treat CF lung infections.

NEUTROPHIL-DERIVED NOREPINEPHRINE SUPPORTS GONOCOCCAL RESISTANCE TO NUTRITIONAL IMMUNITY

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Neisseria gonorrhoeae (Gc) is an urgent public health concern due to increasing cases, rising antibiotic resistance, and severe clinical sequelae. The mucosal inflammatory response in gonorrhea is characterized by the influx of neutrophils that fail to eliminate Gc. One host defense against infection is nutritional immunity: sequestration of essential metals like iron from invading microbes. Gc requires iron for human infection and uses the Ferric uptake regulator (Fur) to control genes involved in iron acquisition and storage. How Gc adapts to low-iron conditions during neutrophil challenge is incompletely understood.

To investigate Gc-neutrophil interactions in low-iron conditions, Chelex-treated defined medium (CDM), which models metal limitation during infection, was conditioned by incubation with primary human neutrophils. Gc growth was monitored by CFU enumeration after incubation in CDM alone, with iron, or with neutrophil conditioning. Unmodified CDM lacked sufficient iron for Gc growth. Neutrophil conditioning of CDM enabled Gc growth.

Neutrophil conditioning of CDM did not increase the iron concentration of the media, measured by ICP-MS. Instead, a growth-promoting factor < 3 kDa was discovered using molecular weight fractionation. Metabolomics identified this factor as the catecholamine hormone norepinephrine. Our results show that norepinephrine is released by neutrophils and promotes Gc growth under iron-limited conditions where growth is otherwise restricted; addition of norepinephrine to unmodified CDM was sufficient to restore Gc growth.

By RNA-seq, norepinephrine promoted an iron starvation response in Gc characterized by de-repression of the gonococcal Fur regulon. A *fur-1* mutant, with reduced Fur activity, grew significantly better in CDM than wild type, highlighting that de-repression of the Fur regulon is advantageous in low-iron conditions. Norepinephrine did not increase growth of the *fur-1* mutant, further implicating Fur in the gonococcal response to norepinephrine. Anaerobic DNA-protein interaction ELISA showed that norepinephrine inhibits Fur binding to the promoter of a Fur-regulated gene.

The neuroendocrine hormone norepinephrine, which is released by primary human neutrophils, promotes Gc growth under iron-limited conditions where growth is otherwise restricted. The mechanism by which norepinephrine supports Gc growth presents an exciting area for discovery; our results suggest that norepinephrine alters Gc iron homeostasis. Insights have been obtained into how Gc uses physiologically relevant cues to persist in the presence of robust immune responses, with potential to inform future drug and vaccine targets.

STREPTOCOCCUS SUIS MANGANESE TRANSPORTER MUTANT AS A LIVE ATTENUATED VACCINE: SAFETY, EFFICACY, AND VIRULENCE REVERSION MECHANISMS

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Streptococcus suis is the leading cause of mortality in piglets and is responsible for severe economic losses in the global pork industry. Severe invasive diseases caused by *S. suis* include sepsis, meningitis, arthritis, and endocarditis. *S. suis* disease prevention is hampered by the lack of safe and efficacious vaccines. In this study, we constructed an *S. suis* live attenuated vaccine candidate lacking the major streptococcal manganese transporter, a known virulence determinant of this organism. The safety and efficacy of this live vaccine were evaluated in swine. Our clinical study results showed that when administered at a dose of 10^{10} CFU, the vaccine strain was safe and efficacious. However, a lower dose of 10^{9} CFU failed to generate significant immune protection. To investigate if an adjuvant could enhance the efficacy of the vaccine at a lower dose, we spiked the vaccine with a polymeric adjuvant and evaluated its performance. Surprisingly, four pigs receiving the adjuvanted vaccine died during the vaccination phase. Pathology, microbiology, and genetic analyses suggested that the vaccine strain reverted to virulence in these animals. Functional genetic analysis found that the vaccine strain acquired compensatory mutations that upregulated the expression of a secondary manganese transporter, which in turn restored the virulence of the vaccine strain. Our results provide a new understanding of *S. suis* host adaptation mechanisms and useful information for the design of future live-attenuated vaccines.

PROXIMITY PROFILING OF THE *LISTERIA* SURFACE REVEALS PATHOGEN CONTROL OF A HOST DEUBIQUITINASE

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Intracellular pathogens must navigate the crowded cellular environment to establish infection. *Listeria monocytogenes* accomplishes this by recruiting host factors to its surface to hijack the host actin cytoskeleton for motility, forming membrane protrusions, and spreading cell-to-cell. While the recruitment of cytoskeletal regulators that drive actin-based motility is well-characterized, the complete array of host factors directly interacting with *Listeria* to regulate other stages of infection remains incompletely understood. To identify the host factors recruited to the *Listeria* surface, we adapted split-TurboID proximity labeling by expressing the N- and C-terminal domains of TurboID on the bacterial surface and host cytosol, respectively. This approach revealed that the host deubiquitinase CYLD is recruited to the *Listeria* surface during infection. Although CYLD was previously shown to promote infection by suppressing autophagy and innate immunity in macrophages, how *Listeria* recruits CYLD and the functional significance of this localization remained unclear. CYLD recruitment appeared pathogen-specific, as it was not observed with other ubiquitinated intracellular bacteria such as *Rickettsia parkeri*. However, co-infection experiments demonstrated that *Listeria* could induce CYLD recruitment to *R. parkeri*, suggesting a *Listeria*-specific factor modified CYLD's behavior. Indeed, we identified this factor as internalin C (InlC), a secreted bacterial effector sufficient to localize CYLD to ubiquitinated bacteria. Structural predictions indicate InlC binds CYLD's second CAP-glycine domain, and deletion of this domain prevents CYLD localization to *Listeria*. Functionally, CYLD knockout in epithelial cells did not affect bacterial replication but significantly impaired *Listeria* spread to neighboring cells, indicating an additional role for CYLD during infection. These findings reveal that *Listeria* employs the secreted effector InlC to manipulate the host-pathogen interactome by recruiting CYLD to the bacterial surface and co-opting CYLD to promote cell-to-cell spread. Current work is focused on investigating the mechanism by which InlC directs CYLD to ubiquitinated substrates, what impact the InlC-CYLD interaction has on CYLD's deubiquitinase activity and substrate specificity, and how these interactions promote bacterial spread. Overall, our work demonstrates how understanding pathogenic control of the host bacterial surface interactome can reveal new insights into how intracellular bacterial pathogens take control of host function.

INVESTIGATING THE ROLE OF CIRCADIAN REGULATION IN CHLAMYDIA PATHOGENESIS USING A SIAH2 KNOCKOUT MOUSE MODEL

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Chlamydia trachomatis is the most common bacterial sexually transmitted infection globally and a leading cause of preventable infertility in women. It often migrates through the female upper reproductive tract, causing inflammation, scarring, and permanent damage to reproductive organs. While antibiotic treatments are effective at clearing the infection, long-term reproductive consequences can persist due to host-pathogen interactions that are not fully understood. Identifying host genetic factors that influence the severity of infection and subsequent reproductive damage is critical. SIAH2, an E3 ubiquitin ligase, is involved in regulating inflammation, cellular metabolism, and circadian rhythm, and may play an unrecognized role in host defense mechanisms against intracellular pathogens like Chlamydia. SIAH2 has also been shown to modulate REV-ERBα, a core component of the circadian clock. Notably, SIAH2 knockout (KO) female mice exhibit sex-specific alterations in metabolism and rhythmic gene expression, particularly in the liver.

In this study, we used a SIAH2 KO mouse model to investigate how circadian disruption and various stages of the estrous cycle influence susceptibility to Chlamydia trachomatis infection. Female mice were infected during the estrous and diestrous phases to assess the impact of the estrous cycle and circadian factors on disease pathogenesis. Preliminary findings suggest that SIAH2 KO mice infected during diestrous exhibit reduced genital tract pathology and inflammation, while those infected during estrous show heightened inflammatory responses. As well as wild-type mice infected at diestrus showed less pathology and inflammation than their KO counterparts. These results indicate that reproductive stage and circadian regulation may interact to shape the host response to Chlamydia, with implications for understanding infection outcomes and optimizing treatment strategies.

LOWER AIRWAY ORAL COMMENSAL EXPOSURE ALTERS HOST-MICROBIOTA RESPONSE TO *STAPHYLOCOCCUS AUREUS* INFECTION IN A PRE-CLINICAL MODEL

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RATIONALE: The lower airway microbiome consists of a fluctuating community of *Streptococcus, Prevotella, and Veillonella.* We explored how host-microbiome interactions alter host susceptibility to low virulent *agr*-deficient *S. aureus*. We hypothesized that the lower airway dysbiosis may alter host-microbiota interactions that select *agr*-deficient strains of *S. aureus*.

METHODS: We used wild-type 9-week-old C57BL6J female mice (IACUC #s16-00032) and intra-tracheally challenged with a 50uL mixture of oral commensals (MOC): *Streptococcus mitis* (1x10^7 cfu/mouse), *Veillonella parvul*a (2.5x10^7 cfu/mouse), and *Prevotella melaninogenica* (2x10^7 cfu/mouse) (PMID 33166473) over one, four, eight, sixteen weeks of intra-tracheal exposures, one exposure per week. Control mice were given PBS. S. aureus (USA300) and agr-deficit *S. aureus* strains (CC8) were grown on CHROMagar, and mice were exposed to a 1:1 intra-tracheal of *S. aureus* (3-9x10^7 cfu/mouse) 2 weeks after MOC. Differences in mortality and *S. aureus* recovery (competition index: CI) were calculated from lavage.

RESULTS: We tested mortality and *Staphylococcus* recovery in mice with chronic MOC, single MOC, and PBS with USA300 strain, which showed decreased mortality with MOC vs. PBS (Kaplan-Meier $p<0.001$) and reduced lower airway *Staphylococcus* recovery (PBS vs. Single MOC $p<0.05$, PBS vs. Chronic MOC $p<0.05$). In a following experiment, we performed a CC8:USA300 CI experiment to understand how dysbiosis affects lower airway resistance to S. aureus. Here, we found that single MOC exposure cleared CC8 ($CI<1.0$), but 16-week MOC cleared USA300 ($CI>1.0$). The protective effect of oral commensal aspiration nadired around 8-weeks.

CONCLUSIONS: Our study establishes that lower airway exposure to human oral commensals results in *Staphylococcus* resistance and altered inflammatory and immune pathways. Our data suggests the *S. aureus* response may be influenced by acute and chronic host-microbiome interactions. Early exposure to MOC may clear for *agr*-deficient S. aureus strains while chronic exposure may clear wild-type USA300 *S. aureus* strains. Further research will assess host-microbiome interactions to evaluate the effect of oral commensals on pathogen mechanisms.

GLP-1R AGONISTS AND PPAR-γ AGONISTS ARE NOVEL THERAPEUTIC APPROACHES TO MODULATE LUNG INFECTION AND ATTENUATE INFLAMMATION IN COPD

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Cigarette smoking-mediated chronic obstructive lung disease (COPD) is a major cause of death globally. There is no cure for COPD other than a lung transplant, and current therapies only manage symptoms and quality of life, highlighting the urgent need for novel treatments. Acute exacerbations in COPD are predominantly caused by microbial infections and air pollutants, with the Gram-negative respiratory pathogen *Pseudomonas aeruginosa* being a significant contributor at the advanced stages of COPD. Pyocyanin, a *P. aeruginosa* redox-active toxin, and cigarette smoke extract (CSE) trigger mucus hypersecretion and inactivate the mucociliary escalator. Previously, we showed that the prototype GLP1R agonist Exenatide, inhibits pyocyanin-induced mucus hypersecretion by activating the GLP1R-PPARγ-PTEN/PTP1B phosphatases signaling pathway. In this study, we compare Exenatide to the next-generation GLP-1R and PPAR-γ agonists for improving airway mucus homeostasis, mucociliary escalator function, and *P. aeruginosa* clearance after chronic pyocyanin and cigarette smoke exposure, followed by airway infection. GLP-1R and PPAR-γ agonists were screened based on their ability to attenuate pyocyanin/CSE-induced oxidative stress and mucus overexpression in 16HBE cells by the Cellular ROS assay and Western blotting, respectively. The ability of these agonists to restore mucociliary escalator function in the air-liquid interface (ALI) culture of small airway epithelial cells (SAECs) was captured by confocal microscopy. Finally, we examined whether these agonists inhibit pyocyanin-induced mucus hypersecretion in C57BL/6 mice. Based on the ROS and western blot screens, we found that the GLP-1R agonist Semaglutide and the PPAR-γ agonist Pioglitazone are the most effective in decreasing mucus biomarker MUC5AC expression and ROS production in response to pyocyanin while Liraglutide and Rosiglitazone are most effective against CSE, both in vitro and in mice. Importantly, these agonists restore mucociliary beat frequency and mucociliary transport inhibited by pyocyanin and CSE. Furthermore, Semaglutide and Pioglitazone attenuated neutrophil, T-helper (Th) 17, and Th2-dominated proinflammatory responses that are the main drivers of mucus hypersecretion as well as *P. aeruginosa* burden in mouse lungs. Similarly, efficacy is also observed with Liraglutide and Rosiglitazone against cigarette smoke-induced mucus hypersecretion, proinflammatory responses, and P. aeruginosa burden. Collectively, our studies suggest new adjunctive therapeutic approaches for COPD by repurposing FDA-approved GLP-1R and PPAR-γ agonists that can be immediately deployed to manage COPD.

PATHOGEN-COMMENSAL INTERACTIONS DRIVE BMP-MEDIATED ARREST OF INTESTINAL REPAIR DURING *VIBRIO CHOLERAE* INFECTION

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To maintain an effective barrier, intestinal progenitor cells must divide at a rate that matches the loss of dead and dying cells. Otherwise, epithelial breaches expose the host to systemic infection by gut-resident microbes. Unlike most pathogens, *Vibrio cholerae* blocks gut tissue renewal by arresting the proliferation of intestinal progenitor cells in the Drosophila model. We find that *V. cholerae* infection activates the growth inhibitor Bone Morphogenetic Protein (BMP) pathway in progenitors. Specifically, a Type VI Secretion System (T6SS)-dependent interaction between *V. cholerae* and gut commensals initiates BMP signaling via host innate immune defenses. Notably, we find that this pathogen-symbiont interaction also activates BMP and arrests cell proliferation in the intestines of the vertebrate zebrafish model. Together, our study highlights how pathogen-commensal interplay engages evolutionarily conserved immune and growth regulators to impact host tissue repair.

UNRAVELING THE IMPACT OF HOST LIPID PIRACY ON STAPHYLOCOCCUS AUREUS SEPSIS

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Staphylococcus aureus (S. aureus) is an opportunistic pathogen that poses significant public health threats worldwide. During colonization and infection of mammalian hosts, S. aureus exploits host-derived macromolecules to support its growth. In this study, we investigated the influence of host lipids on S. aureus during sepsis. We observed that S. aureus can grow in 100% human serum, leading to remodeling of its cell membrane through the incorporation of serum-derived lipids. To confirm that this lipid membrane remodeling occurs in vivo, we developed a sepsis model and established a mass spectrometry-based method to measure the acquisition of host lipids by the bacterium. Our findings revealed that S. aureus isolated from infected tissues in vivo also remodeled their membranes with host-derived lipids, mirroring the changes seen in bacteria grown in human serum. Notably, this incorporation of host lipids occurs rapidly both in vitro and in vivo. To further investigate this adaptive behavior, we optimized a chemically defined medium that recapitulates the lipid composition of human serum, enabling us to study the impact of host lipids on S. aureus biology under controlled conditions. We discovered that the incorporation of host-derived lipids attenuates S. aureus virulence, a phenotype linked to the inactivation of at least two major two-component regulatory systems that control the expression of numerous virulence factors. Overall, our work highlights a key early adaptation of S. aureus during mammalian infection and provides new insights into the interplay between metabolism and bacterial pathogenesis.

VIBRIO CHOLERAE BIOFILMS USE MODULAR ADHESINS WITH GLYCAN-TARGETING AND EXOPOLYSACCHARIDE-RECOGNITION DOMAINS FOR HOST COLONIZATION

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Bacterial biofilms are surface-attached communities enclosed by an extracellular matrix that promote both environmental persistence and host infection. In most biofilm-forming species, exopolysaccharides (EPSs) play a dominant role in maintaining biofilm structural integrity; however, our understanding of the chemical and physicochemical properties of EPSs remains limited. In addition, matrix proteins have recently been suggested to play indispensable roles in biofilm formation, particularly in biofilm-to-surface adhesion and crosslinking of EPS. Despite years of research, we still lack a molecular understanding of protein-EPS interactions and how these interactions shape the architecture of the biofilm. Our key question is: in a sea of proteins and polysaccharides in the host, how can secreted matrix proteins specifically recognize their cognate exopolysaccharides?

We study this question using *Vibrio cholerae* (*Vc*), the causal agent of pandemic cholera, as the model biofilm-forming organism. Two partially redundant proteins, Bap1 and RbmC, are known to facilitate the adhesion of *Vc* biofilms to various surfaces and to crosslink Vibrio polysaccharide (VPS), the major structural component of *Vc* biofilms. Combining tools from bacterial genetics, glycobiology, biochemistry, single-cell imaging, materials characterization, and structural biology, we show how the two matrix proteins in *Vc* contribute to biofilm adhesion and structural integrity, and how the unique VPS chemical structure enables recognition and tight binding by the β-propeller domain shared by the two adhesins. We further demonstrate that this binding controls the VPS molecular configuration, matrix organization, and mechanics of the *Vc* biofilms. Finally, we will show preliminary results highlighting importance of our biochemical finding in the host context. Overall, our results contribute to our understanding of biofilms in general, leading to new strategies for reducing biofilm-related infections in the long run.

INVESTIGATING THE GENOMICS AND TRANSMISSION OF *STAPHYLOCOCCUS AUREUS* BLOODSTREAM INFECTIONS IN PEOPLE WHO INJECT DRUGS.

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Background: Injection drug use (IDU) is an ongoing health crisis that is complicated by *Staphylococcus aureus* bloodstream infections (IDU-BSI). The epidemiology, transmission, and biology of *S. aureus* IDU-BSI must be defined to develop strategies to limit IDU associated morbidity and mortality.
Methods: We performed whole genome long and short read sequencing of *S. aureus* isolates from the blood and skin of 44 *S. aureus* BSI patients with and 47 patients without a history of IDU, and 32 syringes collected from the community between 2022-2024 from a large hospital and city in St. Louis, MO, USA. Hybrid assemblies of *S. aureus* were generated using autocycler, polypolish, and pypolca. Clonality was assigned with Snippy using a core genome alignment cutoff of 15 SNPs.
Results: We show that 24/24 community syringe isolates collected before 2024 and 1/8 after 2024 form a clonal cluster with isolate differences of less than 4 SNPs. 6/8 2024 isolates form a second clonal cluster, with 1 remaining 2024 isolate unrelated to either cluster. Both strain clusters from community syringes were related to *S. aureus* bloodstream isolates isolated from hospitalized patients with a history of IDU. Among hospitalized patients *S. aureus* was isolated from 11 IDU and 18 control patient skin swabs. 8/11 IDU and 12/18 control patients shared *S. aureus* between their skin and blood. 15/44 IDU patients shared isolates with other unrelated IDU patients. All incidences of BSI clonality involving control patients (22 control – IDU, 6 control – control), can be traced to hospital acquired infections (HAI) experienced by the control patient.
Conclusions: In a community with limited access to needle exchange, we identify clonal and temporally dependent expansion of *S. aureus* isolated from syringes and between IDU patients. These data suggest that drug paraphernalia may be acting as fomites of *S. aureus* transmission, and that dominant strains in the community may be replaced over time. Furthermore, shared *S. aureus* BSI isolates between IDU patients and control patients with a history of HAI indicate that non-IDU patients may be acquiring IDU-related *S. aureus* strains through healthcare encounters.

SEQUENTIALLY ACTIVATED DEATH COMPLEXES REGULATE PYROPTOSIS IN RESPONSE TO *YERSINIA* BLOCKADE OF IMMUNE SIGNALING

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The innate immune system defends against bacterial pathogens initiating a conserved cell death pathway in response to pathogen associated molecular patterns (PAMPS). Pathogenic *Yersinia* species inject a variety of virulence proteins, including YopJ, which inhibits host inflammatory gene expression by blocking the activity of IKK. This blockade triggers caspase-8-dependent cell death that is accompanied by the activation of caspase-1 via a mechanism that is independent of known canonical inflammasome components. Here we demonstrate that caspase-1 activation by caspase-8 requires caspase-8 dimerization, auto processing, and catalytic activity. We further find that caspase-1 catalytic activity is also required for its own processing downstream of caspase-8, suggesting that caspase-8 mediates caspase-1 activation by acting both as an enzyme and as a scaffold. Furthermore, while the inflammasome adaptor protein ASC is dispensable cell death and capsase-1 activation in response to YopJ blockade of IKK, IL-1β maturation and release in the setting of YopJ-induced cell death required components of the canonical NLRP3 inflammasome. Notably, assembly of ASC specks followed the activation of caspase-8 and was reduced in the absence of GSDMD, suggesting that it occurs downstream of caspase-8 and gasdermin D activation. Altogether, this work demonstrates that caspase-8 and ASC participate in functionally interconnected but distinct complexes to mediate cell lysis and IL-1β release in response to pathogen blockade of innate immune signaling.

GUT MICROBIAL INTERACTION NETWORKS UNDERPIN AUTOIMMUNITY TO AN IMMUNE-PRIVILEGED EXTRAINTESTINAL SITE

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Autoimmune uveitis is a sight-threatening inflammation driven by retina-specific T cells. Using a transgenic mouse model of spontaneous autoimmune uveitis, we demonstrated that commensal gut flora can provide innate and adaptive immune stimuli that trigger ocular autoimmunity. We now examined whether uveitis-promoting microbes are present in human gut, by colonizing germ-free R161H mice with fecal microbiota from human donors. Human gut commensals supported uveitis development in these mice, albeit with slightly lower disease scores and a reduced gut Teff/Treg ratio than did conventional mouse flora. "Humanized" gnotobiotic mice retained a distinct but simplified gut microbiota compared to their original donor. Higher uveitis scores correlated with a greater microbial diversity and expansion of specific bacterial taxa (Akkermansia), together with lower levels of short-chain fatty acids (SCFAs) and a lower abundance of SCFA-producing bacteria. Furthermore, association with human flora "spiked" with Akkermansia resulted in higher uveitis scores and a more prominent Th1 effector response in the gut. Notably, the negative correlation between Akkermansia (Verrucomicrobiae) and Firmicutes (Clostridia) was found in fecal microbiota from uveitis patients but not from healthy controls. We suggest that Akkermansia may promote uveitis in vivo by outcompeting SCFA-producing microbes and by promoting a Type 1 inflammatory response. Our data revealed a formerly uncharted microbial interaction network in the mammalian gut ecosystem that support autoimmunity to an immune-privileged extraintestinal site. Both stimulatory and inhibitory microbial components identified from our "humanized" spontaneous autoimmune uveitis model represent potential candidates for microbiome-directed therapeutic intervention.

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CODE OF CONDUCT FOR ALL PARTICIPANTS IN CSHL MEETINGS

Cold Spring Harbor Laboratory (CSHL or the Laboratory) is dedicated to pursuing its twin missions of research and education in the biological sciences. The Laboratory is committed to fostering a working environment that encourages and supports unfettered scientific inquiry and the free and open exchange of ideas that are the hallmarks of academic freedom. To this end, the Laboratory aims to maintain a safe and respectful environment that is free from harassment and discrimination for all attendees of our meetings and courses as well as associated support staff, in accordance with federal, state and local laws.

Consistent with the Laboratory's missions, commitments and policies, the purpose of this Code is to set forth expectations for the professional conduct of all individuals participating in the Laboratory's meetings program, both in person and virtually, including organizers, session chairs, invited speakers, presenters, attendees and sponsors. This Code's prohibition against discrimination and harassment is consistent with the Laboratory's internal policies governing conduct by its own faculty, trainees, students and employees.

By registering for and attending a CSHL meeting, either in person or virtually, participants agree to:

1. Treat fellow meeting participants and CSHL staff with respect, civility and fairness, without bias based on sex, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, nationality or national origin, citizenship status, disability status, veteran status, marital or partnership status, age, genetic information, or any other criteria prohibited under applicable federal, state or local law.

2. Use all CSHL facilities, equipment, computers, supplies and resources responsibly and appropriately if attending in person, as you would at your home institution.

3. Abide by the CSHL Meeting Alcohol Policy (*see below)*.

Similarly, meeting participants agree to refrain from:

1. Harassment and discrimination, either in person or online, in violation of Laboratory policy based on actual or perceived sex, pregnancy status, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, creed, nationality or national origin, immigration or citizenship status, mental or physical disability status, veteran status, military status, marital or partnership status, marital or partnership status, familial status, caregiver status, age, genetic information, status as a victim of domestic violence, sexual violence, or stalking, sexual reproductive health decisions, or any other criteria prohibited under applicable federal, state or local law.

2. Sexual harassment or misconduct.

3. Disrespectful, uncivil and/or unprofessional interpersonal behavior, either in person or online, that interferes with the working and learning environment.

4. Misappropriation of Laboratory property or excessive personal use of resources, if attending in person.

BREACHES OR VIOLATIONS OF THE CODE OF CONDUCT

Cold Spring Harbor Laboratory aims to maintain in-person and virtual conference environments that accord with the principles and expectations outlined in this Code of Conduct. Meeting organizers are tasked with providing leadership during each meeting, and may be approached informally about any breach or violation. Breaches or violations should also be reported to program leadership in person or by email:

- Dr. David Stewart, Grace Auditorium Room 204, 516-367-8801 or x8801 from a campus phone, stewart@cshl.edu
- Dr. Charla Lambert, Hershey Laboratory Room 214, 516-367-5058 or x5058 from a campus phone, clambert@cshl.edu

Reports may be submitted by those who experience harassment or discrimination as well as by those who witness violations of the behavior laid out in this Code.

The Laboratory will act as needed to resolve the matter, up to and including immediate expulsion of the offending participant(s) from the meeting, dismissal from the Laboratory, and exclusion from future academic events offered by CSHL.

If you have questions or concerns, you can contact the meeting organizers, CSHL staff.

For meetings and courses funded by NIH awards:

Participants may contact the Health & Human Services Office for Civil Rights (OCR). See this page for information on filing a civil rights complaint with the OCR; filing a complaint with CSHL is not required before filing a complaint with OCR, and seeking assistance from CSHL in no way prohibits filing complaints with OCR. You may also notify NIH directly about sexual harassment, discrimination, and other forms of inappropriate conduct at NIH-supported events.

For meetings and courses funded by NSF awards:

Participants may file a complaint with the NSF. See this page for information on how to file a complaint with the NSF.

Law Enforcement Reporting:

- For on-campus incidents, reports to law enforcement can be made to the Security Department at 516-367-5555 or x5555 from a campus phone.
- For off-campus incidents, report to the local department where the incident occurred.

In an emergency, dial 911.

DEFINITIONS AND EXAMPLES

Uncivil/disrespectful behavior is not limited to but may take the following forms:

- Shouting, personal attacks or insults, throwing objects, and/or sustained disruption of talks or other meeting-related events

Harassment is any unwelcome verbal, visual, written, or physical conduct that occurs with the purpose or effect of creating an intimidating, hostile, degrading, humiliating, or offensive environment or unreasonably interferes with an individual's work performance. Harassment is not limited to but may take the following forms:

- Threatening, stalking, bullying, demeaning, coercive, or hostile acts that may have real or implied threats of physical, professional, or financial harm
- Signs, graphics, photographs, videos, gestures, jokes, pranks, epithets, slurs, or stereotypes that comment on a person's sex, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, nationality or national origin, citizenship status, disability status, veteran status, marital or partnership status, age, genetic information, or physical appearance

Sexual Harassment includes harassment on the basis of sex, sexual orientation, self-identified or perceived sex, gender expression, gender identity, and the status of being transgender. Sexual harassment is not limited to sexual contact, touching, or expressions of a sexually suggestive nature. Sexual harassment includes all forms of gender discrimination including gender role stereotyping and treating employees differently because of their gender. *Sexual misconduct* is not limited to but may take the following forms:

- Unwelcome and uninvited attention, physical contact, or inappropriate touching
- Groping or sexual assault
- Use of sexual imagery, objects, gestures, or jokes in public spaces or presentations
- Any other verbal or physical contact of a sexual nature when such conduct creates a hostile environment, prevents an individual from fulfilling their professional responsibilities at the meeting, or is made a condition of employment or compensation either implicitly or explicitly

MEETING ALCOHOL POLICY

Consumption of alcoholic beverages is not permitted in CSHL's public areas other than at designated social events (wine and cheese reception, picnic, banquet, etc.), in the Blackford Bar, or under the supervision of a licensed CSHL bartender.

No provision of alcohol by meeting sponsors is permitted unless arranged through CSHL.

Meeting participants consuming alcohol are expected to drink only in moderation at all times during the meeting.

Excessive promotion of a drinking culture at any meeting is not acceptable or tolerated by the Laboratory. No meeting participant should feel pressured or obliged to consume alcohol at any meeting-related event or activity.

VISITOR INFORMATION

EMERGENCY (to dial outside line, press 3+1+number)	
CSHL Security	**516-367-8870 (x8870 from house phone)**
CSHL Emergency	**516-367-5555 (x5555 from house phone)**
Local Police / Fire	**911**
Poison Control	**(3) 911**

CSHL SightMD Center for Health and Wellness ***(call for appointment)*** Dolan Hall, East Wing, Room 111 **cshlwellness@northwell.edu**	**516-422-4422** **x4422 from house phone**
Emergency Room **Huntington Hospital** 270 Park Avenue, Huntington	**631-351-2000**
Dentists Dr. William Berg Dr. Robert Zeman	 **631-271-2310** **631-271-8090**
Drugs - 24 hours, 7 days Rite-Aid 391 W. Main Street, Huntington	**631-549-9400**

GENERAL INFORMATION

Meetings & Courses Main Office

Hours during meetings: M-F 9am – 9pm, Sat 8:30am – 1pm
After hours – See information on front desk counter
For assistance, call Security at 516-367-8870
(x8870 from house phone)

Dining, Bar

Blackford Dining Hall (main level):
Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00
Blackford Bar (lower level): 5:00 p.m. until late

House Phones

Grace Auditorium, upper / lower level; Cabin Complex; Blackford Hall; Dolan Hall, foyer

Books, Gifts, Snacks, Clothing

CSHL Bookstore and Gift Shop
516-367-8837 (hours posted on door)
Grace Auditorium, lower level.

Computers, E-mail, Internet access

Grace Auditorium
Upper level: E-mail and printing in the business center area
WiFi Access: GUEST (no password)

Announcements, Message Board Mail, ATM, Travel info

Grace Auditorium, lower level

Russell Fitness Center
Dolan Hall, east wing, lower level
PIN#: **(On your registration envelope)**

Laundry Machines
Dolan Hall, lower level

Photocopiers, Journals, Periodicals, Books
CSHL Main Library
Open 24 hours (with PIN# or CSHL ID)
Staff Hours: 9:00 am – 9:00 pm
Use PIN# (On your registration envelope) to enter Library
See Library staff for photocopier code.
Library room reservations (hourly) available on request between 9:00 am – 9:00 pm

Swimming, Tennis, Jogging, Hiking
June–Sept. Lifeguard on duty at the beach. 12:00 noon–6:00 p.m.
Two tennis courts open daily.

Local Interest

Fish Hatchery	631-692-6758
Sagamore Hill	516-922-4788
Whaling Museum	631-367-3418
Heckscher Museum	631-351-3250
CSHL DNA Learning Center	x 5170

New York City
Helpful tip -
Take CSHL Shuttle OR Uber/Lyft/Taxi to Syosset Train Station
Long Island Railroad to Penn Station
Train ride about one hour.

TRANSPORTATION

Limo, Taxi

Syosset Limousine	516-364-9681
Executive Limo Service	516-826-8172
Limos Long Island	516-400-3364
Syosset Taxi	516-921-2141
Orange & White Taxi	631-271-3600
Uber / Lyft	

Trains

Long Island Rail Road	718-217-LIRR (5477)
Amtrak	800-872-7245
MetroNorth	877-690-5114
New Jersey Transit	973-275-5555

CSHL's Green Campus

Cold Spring Harbor Laboratory is pledged to operate in an environmentally responsible fashion wherever possible. In the past, we have removed underground oil tanks, remediated asbestos in historic buildings, and taken substantial measures to ensure the pristine quality of the waters of the harbor. Water used for irrigation comes from natural springs and wells on the property itself. Lawns, trees, and planting beds are managed organically whenever possible. And trees are planted to replace those felled for construction projects.

Two areas in which the Laboratory has focused recent efforts have been those of waste management and energy conservation. The Laboratory currently recycles most waste. Scrap metal, electronics, construction debris, batteries, fluorescent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a "single stream waste management" system, removing recyclable materials and sending the remaining combustible trash to a cogeneration plant where it is burned to provide electricity, an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced with high efficiency fluorescent fixtures, and thousands of incandescent bulbs throughout campus have been replaced with compact fluorescents. The Laboratory has also embarked on a project that will replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new ways in which we can reduce our environmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory's efforts can be improved. This book, for example, is printed on recycled paper.

Cold Spring Harbor Laboratory Bookstore & Gift Shop

Main campus, lower level of Grace Auditorium

Store Hours

Did you miss your chance to shop at the CSHL Bookstore and Gift Shop during the conference?

No problem! Visit our Online Bookstore and Gift Shop.

It's a great way to bring home a piece of the experience!

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CSHL Campus Map

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