# 56th ANNUAL MIDWEST STUDENT BIOMEDICAL RESEARCH FORUM

## Saturday, March 8, 2025

## ROOM 3040

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|---|---|---|
| 1:30 p.m. | O-19 | ACTIVATION OF THE INTERFERON RESPONSE IN UNINFECTED BYSTANDER CELLS FOLLOWING CHLAMYDIA TRACHOMATIS INFECTION – IMPLICATIONS ON DISSEMINATION DYNAMICS<br>Presenter: Francis Fontanilla |
| 1:45 p.m. | O-84 | EVALUATING THE SIGNIFICANCE OF TRP OPERON REGULATION IN CHLAMYDIA TRACHOMATIS<br>Presenter: Tyler Zimmerman |
| 2:00 p.m. | O-81 | HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN H3 REGULATES HIV-1 INFECTION<br>Presenter: Kayla Weldon |
| 2:15 p.m. | O-82 | DNA METHYLTRANSFERASE ACTIVITY DIFFERENTIALLY PROGRAMS LEUKOCYTE RESPONSES TO S. AUREUS<br>Presenter: Leann Xu |
| 2:30 p.m. | O-66 | RAPID AND SENSITIVE DETERMINATION OF RESIDUAL PRION INFECTIVITY FROM PRION DECONTAMINATED SURFACES<br>Presenter: Sara Simmons |
| 2:45 p.m. | O-36 | DEVELOPMENT OF IN VITRO ORGANOTYPIC SKIN TO STUDY THE LYME DISEASE PATHOGEN<br>Presenter: Jaxon Kramer |
| 3:00 p.m. | O-33 | KEEP (PI)CALM AND CARRY ON: MECHANISMS OF GOLGI TRAFFICKING MANIPULATION BY CHLAMYDIA TRACHOMATIS<br>Presenter: Haley Knowles |
| 3:15 p.m. | O-30 | IDENTIFICATION OF METHYLATION ON THE CERE OF NAEGLERIA SPECIES<br>Presenter: Niklas Johnson |
| 3:30 p.m. | O-26 | THE SPX REDOX SWITCH CONTROLS CYSTINE UPTAKE AND TOXICITY IN STAPHYLOCOCCUS AUREUS UNDER DISULFIDE STRESS<br>Presenter: Abigail Hall |
| 3:45 p.m. | O-09 | INVESTIGATING THE ROLE OF S. AUREUS CITRATE METABOLISM DURING PROSTHETIC JOINT INFECTION<br>Presenter: Nichole Brandquist |
| **4:00 p.m.** | | **ADJOURN** |

# ACTIVATION OF THE INTERFERON RESPONSE IN UNINFECTED BYSTANDER CELLS FOLLOWING CHLAMYDIA TRACHOMATIS INFECTION – IMPLICATIONS ON DISSEMINATION DYNAMICS

Francis Fontanilla, Rey Carabeo, and Amanda Brinkworth (University of Nebraska Medical Center, Omaha, NE)

**Background, Significance, and Hypothesis:** *Chlamydia trachomatis (Ctr)* is an obligate intracellular pathogen and is the primary cause of bacterial sexually transmitted infections (STI). Although often asymptomatic, infections could progress to the upper genital tract and may lead to serious reproductive health sequelae such as pelvic inflammatory disease, ectopic pregnancy, and even infertility if left untreated. With 1.6 million cases nationwide and over $690 million in direct lifetime costs, *Ctr* is considered a major public health burden. Some individuals spontaneously clear infections which have been attributed to host adaptive immunity. However, studies also show that infections can persist, and reinfections occur suggesting that long-term protective immunity is partial at best. Although the adaptive immune response to *Ctr* infection is well-characterized, how active infection influences host innate immunity particularly at the *Ctr*-epithelium interface remains largely unexplored. Moreover, characterization of host responses during early *Ctr* infection of the cervical epithelium with uninfected bystander cells present are often overlooked in favor of infection models that utilize a fully infected epithelial monolayer where samples are collected at a single timepoint post infection. This further underscores the need to investigate host responses across multiple timepoints during infection which may have implications in *Ctr* survival and spread. The cervical epithelium sits at the center of *Ctr*-host interaction as it is the primary site of infection. Soluble factors including interferons (IFNs) are produced in abundance in the infection microenvironment. Epithelial-related IFNs such as IFNβ and IFNλ drive the expression of IFN-stimulated genes (ISGs) through the JAK-STAT pathway in an autocrine and paracrine manner. Since most ISGs are putative antimicrobials, the cumulative epithelial response is generally antimicrobial which aids in pathogen restriction. Thus, cytokine signaling in infected epithelial cells is often a target for subversion by pathogens. We recently showed that *Ctr* can dampen the epithelial IFN response of a fully infected epithelial monolayer. This is in contrast to what is known in the field as *Ctr*-epithelial host immunobiology is commonly viewed as pro-inflammatory. To reconcile the observed attenuated epithelial IFN response with the current notion of a pro-inflammatory *Ctr*-epithelial interaction, we hypothesize that bystander cells are crucial in shaping the cytokine environment, and dysregulation of the epithelial IFN response may have direct consequences in *Ctr* dissemination.

**Experimental Design:** To address this, we monitored the response of a sparsely infected endocervical epithelial monolayer over a period of 72 hours. In determining the response at the monolayer population level, protein lysates were collected every 24 hours and probed for markers of activated epithelial IFN response such as phospho-STAT1 and the expression of the downstream ISG effector IRF1. Moreover, individual cell responses within the monolayer was also assessed using immunofluorescence microscopy.

**Data and Results:** Here, we found that *Ctr* downmodulated the expression of *IFNβ1* and *IFNλ1* in a fully infected endocervical epithelial monolayer. This corresponded with decreased STAT1 activation and lower expression of ISGs like GBP1 and IRF1. In the monolayer infected at low multiplicity, increased levels of activated STAT1 and IRF1 were observed at 48 hpi, but unexpectedly decreased at 72 hpi. Using a quantitative imaging-based assay, our results show two contrasting subpopulations in terms of infection and nuclear IRF1 levels. While infected cells exhibited an attenuated phenotype, consistent with the fully infected monolayer studies, uninfected bystander cells had higher levels of nuclear IRF1. We predict that pre-activation of bystander cells via paracrine signaling will lead to the induction of several potentially novel IRF1-regulated anti-chlamydial ISGs that could influence infection dissemination.

**Conclusion:** Collectively, our results show that *Ctr* infection of cervical epithelial monolayer triggers a transient immune response with maximal activation at 48 hpi. Although *Ctr* can actively attenuate this response in infected cells at this timepoint, uninfected bystander cells remain activated and may induce a different transcriptomic profile relative to infected cells. This supports our hypothesis that activated uninfected bystander cells may influence the cytokine milieu in the infection microenvironment. This may have major implications on how the epithelial monolayer responds to secondary *Ctr* infection. Furthermore, we continue to determine how this differentially activated state in the monolayer will affect *Ctr* growth and spread in the endocervical epithelium.

# EVALUATING THE SIGNIFICANCE OF TRP OPERON REGULATION IN CHLAMYDIA TRACHOMATIS

Tyler Zimmerman, Liam Caven, Nick Pokorzynski, and Rey Carabeo (UNMC Omaha, NE)

**Background, Significance, Hypothesis:** *Chlamydia trachomatis* lacks the ability to synthesize tryptophan from glycolysis. Its tryptophan auxotrophy is alleviated by host tryptophan pool, or synthesis via the salvage pathway, involving the enzyme tryptophan synthase. Of particular interest is the tight transcriptional regulation of the truncated *trp* operon of *C. trachomatis*. Transcription is regulated jointly by the repressors TrpR and YtgR, the former binding to the major promoter ($P_{trp}$), and the latter binding to the alternative promoter ($P_{trpBA}$). In addition, we determined that transcriptional derepression at both promoters occurs with different kinetics through simulations ran in a mathematical modeling framework. Derepression by TrpR occurs during transient (<4h) tryptophan starvation, while YtgR derepression required longer starvation to inhibit *de novo* translation along with repressor turnover. This supports the notion of both repressors having distinct, nonredundant roles in the regulation of the truncated *trp* operon. We therefore hypothesize that both TrpR and YtgR are necessary for adapting to tryptophan limiting conditions.

**Experimental Design:**
To evaluate this hypothesis, we needed a mechanism by which we could isolate the effects of each repressor under tryptophan limiting conditions. We found that the tryptophan analogue 5-methyltryptophan (5-MT) could maintain TrpR repression under such conditions. We analyzed the effects *in vitro* of different tryptophan starvation durations both with and without 5-MT, from the perspectives of *trp* operon transcription levels and *Chlamydia trachomatis* inclusion development measured by inclusion size.

Our laboratory has also previously published data using a YtgR-YYF plasmid-containing strain of *C. trachomatis*. The plasmid removes the triple tryptophan motif (WWW) from YtgR and swaps it for YYF. This removes the translational-attenuative effect that low tryptophan conditions have on YtgR production, thereby making its translation independent of tryptophan. Since this strain effectively shuts off the tryptophan regulation of YtgR, we plan to use it to elucidate the importance of YtgR derepression on adapting Chlamydia trachomatis to tryptophan limiting conditions.

**Data and Results:** Inhibiting derepression of TrpR by treatment with 5-MT did not affect *C. trachomatis* inclusion development during transient tryptophan starvation. This is despite the observed >90% repression of transcription of the *trp* operon. However, despite similar levels of transcriptional repression observed with 5-MT treatment during prolonged tryptophan starvation, inclusion development was significantly reduced.

**Conclusion:** Our results demonstrated that TrpR derepression is important during prolonged tryptophan starvation. This supports our hypothesis that TrpR derepression in *Chlamydia trachomatis* is necessary for adaptability to tryptophan limiting conditions. Further research into the impact of interferon-gamma (IFN-γ)-mediated tryptophan starvation rather than direct media removal of tryptophan is warranted as it is more physiologically relevant and delivers stressors beyond tryptophan depletion. Tryptophan depletion may therefore serve as the trigger that allows *C. trachomatis* to adapt to IFN-γ-mediated stressors, as YtgR has genome-wide binding capabilities. We can assess this hypothesis via the use of 1-methyltryptophan (L-1MT). L-1MT prevents only the tryptophan-catabolizing effect of IFN-γ, while leaving all other stressors in place, which would therefore remove the tryptophan depletion “signal” which might be necessary for *C. trachomatis* adaptability.

# HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN H3 REGULATES HIV-1 INFECTION

Kayla M. Weldon, and Michael A. Belshan, Creighton University School of Medicine, Omaha, Nebraska

## Background

Human Immunodeficiency Virus (HIV), the etiologic agent of acquired immunodeficiency syndrome (AIDS), is a member of the lentivirus subfamily of retroviruses. Retroviruses are characterized by undergoing reverse transcription after entry to convert the RNA genome to double-stranded DNA, as well as integrating their DNA genome into the host cell genome. Like other viruses, HIV-1 is an obligate intracellular parasite that requires host cell factors and machinery for productive replication.

## Significance of Problem

Approximately 39 million people globally were living with HIV at the end of 2022, and 630,000 AIDS related deaths occurred. HIV infection can be suppressed to undetectable levels with anti-retroviral therapy (ART). HIV infection is incurable due to the existence of reservoirs of infected cells including both latently infected cells as well as cells persistently replicating HIV at low levels. A “cure” for HIV will require elimination of these latent reservoirs and suppression of ongoing replication. Identifying novel pathways and mechanisms that regulate HIV infection may result in discovery of novel strategies to block virus replication and impair or eliminate HIV persistence in people living with HIV.

## Hypothesis, Problem or Question

Changes in the expression or function of heterogeneous nuclear ribonucleoprotein H3 (hnRNPH3) will modulate the susceptibility of cells to HIV-1 infection. Identifying this factor as a cofactor of HIV infection will inform strategies to inhibit HIV replication through targeted drug treatments.

## Experimental Design

RNA was isolated from seven cell lines with high or low susceptibility to HIV-1 infection and total RNA sequencing was performed, identifying hnRNPH3 as a top candidate gene. The differential expression of hnRNPH3 was validated by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) using gene specific primers (IDTDNA). Expression was measured relative to a control gene (GAPDH) using the $\Delta$ct/$\Delta$ct method. Protein expression of individual factors was determined by quantitative western blot using GAPDH as a control. Over-expression of hnRNPH3 was achieved by pre-transfection of a plasmid expression vector 24 hours prior to infection. Stable knockdown of hnRNPH3 was achieved by lentiviral transduction of shRNAs and selection with puromycin. Single round infections were performed using luciferase reporter viruses pseudotyped with various viral envelopes. Infections were quantified by luciferase assays and normalized to total protein levels. Over-expression and knockdown of gene expression were confirmed by immunoblot. All statistics were performed using GRAPHPAD PRISM Software version 10.1.

## Results/Data

hnRNPH3 expression was initially evaluated using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Results indicated that poorly susceptible cells generally exhibited lower levels of hnRNPH3 expression, suggesting a potential correlation between hnRNPH3 expression and susceptibility to infection. Immunoblot analyses of hnRNPH3 expression across highly and poorly susceptible cell lines yielded inconclusive results, failing to provide consistent evidence of differential protein expression levels. Functional studies examining the role of hnRNPH3 in HIV-1 infection showed that overexpression of hnRNPH3 in cells did not significantly alter the efficiency of HIV-1 transduction, implying that increased hnRNPH3 levels may not enhance viral infectivity. Conversely, stable knockdown of hnRNPH3 led to a measurable decrease in viral transduction efficiency using an HIV-1 with a luciferase reporter construct (HIV-Luc) and pseudotyped with the vesicular stomatitis virus glycoprotein (VSV-G) envelope. This reduction was not limited to HIV-1, as the transduction efficiencies of murine leukemia virus (MLV) pseudotyped with VSVg and HIV pseudotyped with the amphotropic murine leukemia virus envelope (aMLV) were also diminished in hnRNPH3 knockdown cells.

## Conclusions

Cell lines with differential susceptibility to HIV-1 infection display distinct patterns of gene expression. The expression of hnRNPH3 was discovered to correlate with susceptibility to HIV-1 transduction. Stable knockdown of hnRNPH3 lead to a decrease in viral transduction. These data suggest hnRNPH3 is required for efficient HIV-1 infection as cell susceptibility to HIV was reduced when hnRNPH3 expression was knocked down. Results thus far with single-round infection assays with various pseudotyped viruses indicate that knockdown of hnRNPH3 is non-specifically affecting an early step of virus infection, possibly viral entry. Effort is underway to knockdown hnRNPH3 in T-cells to examine the role of hnRNPH3 in the context of full HIV-1 replication.

# DNA METHYLTRANSFERASE ACTIVITY DIFFERENTIALLY PROGRAMS LEUKOCYTE RESPONSES TO *S. AUREUS*

Leann Xu, Zachary Van Roy, and Tammy Kielian

Department of Pathology, Microbiology, and Immunology University of Nebraska Medical Center, Omaha, Nebraska

**Background:** *Staphylococcus aureus* (*S. aureus*) is a Gram-positive pathogen and the leading cause of craniotomy infection. DNA methyltransferases (DNMTs) facilitate the covalent addition of a methyl group to cytosine or adenine within the DNA polymer, leading to reduced chromatin accessibility and subsequent gene silencing. Our laboratory previously conducted a high-throughput screen of an epigenetic compound library, identifying DNA methylation as a key regulator of inflammatory responses to *S. aureus*, particularly IL-10 production, which can have harmful effects during craniotomy infection. This study interrogated the role of DNMTs on cytokine production and T cell proliferation in response to *S. aureus*. A mouse model of craniotomy infection was utilized to examine the effects of a global (pan-DNMT) inhibitor on bacterial growth and leukocyte infiltrates. Future studies will investigate the methylation status of leukocytes at the infection site vs. peripheral circulation to better understand how they are maladaptively programmed during craniotomy infection.

**Significance of Problem:** Craniotomies are among the most frequently performed neurosurgical procedures, involving the removal of a segment of the skull (bone flap) to provide access to the brain to resolve various pathologies, such as the removal of brain tumors or epileptic foci. Infection is one of the most common and severe complications following a craniotomy, occurring in 1-7% of cases, with *S. aureus* being the most common causative agent. Understanding how *S. aureus* induces epigenetic changes in leukocytes will provide insights into potential immunomodulatory treatments for craniotomy infection.

**Question:** How do leukocyte-pathogen interactions alter DNMT activity to impact craniotomy infection outcome?

**Experimental Design and Results:** To examine the effects of DNMT inhibition on cytokine production, specifically IL-10, neutrophils and anti-inflammatory granulocytic myeloid-derived suppressor cells (G-MDSCs) were exposed to either heat-killed *S. aureus* (HKSA) or live bacteria, the latter to evaluate the effect of virulence factors produced by viable organisms. Our results indicated that DNMTs exhibit effects that are specific to cell type and bacterial state. For example, when exposed to HKSA, a global (pan-DNMT) inhibitor decreased IL-10 production by G-MDSCs, whereas cytokine levels were increased in neutrophils (one-way ANOVA). This observation did not extend to live *S. aureus*, where DNMT-3b inhibition significantly reduced IL-10 production by both neutrophils and G-MDSCs (one-way ANOVA). Next, T cell proliferation assays were performed to examine how DNMTs impact neutrophil function. Inhibition of DNMT-3a in neutrophils decreased T cell proliferation (one-way ANOVA) suggesting that changes in DNA methylation status are critical for PMN-T cell interactions. To investigate the functional impact of DNMTs during *S. aureus* craniotomy infection, mice received daily intraperitoneal injections of a pan-DNMT inhibitor (2.5mg/kg/day) and sacrificed at day 7 post-infection. Mice receiving pan-DNMT inhibitor had elevated bacterial burden in the galea compartment concomitant with decreased neutrophil and increased monocyte influx into infected tissues compared to vehicle treated animals (one-way ANOVA). Collectively, these results suggest that inhibiting DNA methylation shifts the tissue microenvironment to a more anti-inflammatory state to promote craniotomy infection.

**Conclusions:** These data indicate that DNMT activity has a significant impact on leukocyte activation and function that in some cases is influenced by specific DNMT isoforms. Further studies will utilize Nanopore™ and methylation microarray technologies to reveal how *S. aureus* alters the methylation landscape in neutrophils and G-MDSCs.

# RAPID AND SENSITIVE DETERMINATION OF RESIDUAL PRION INFECTIVITY FROM PRION DECONTAMINATED SURFACES

Simmons SM, Payne VP, Hrdlicka JG, Taylor J, Yuan Qi and Bartz JC (Creighton University School of Medicine, Omaha, Nebraska)

**Background, Significance and Problem:** Prion diseases are transmissible neurodegenerative diseases that lack either a treatment or cure and are uniformly fatal. Prion diseases are caused by $PrP^{Sc}$, the misfolded and pathogenic form of the normal, cellular form of the prion protein, $PrP^{C}$. Prions can bind to surfaces and resist common disinfection techniques. Cases of iatrogenic infection have occurred historically in the context of contaminated surgical instruments. More recent cases of occupationally linked transmission have occurred in laboratory workers handling prion contaminated samples. A method to assess the contamination state of laboratory and clinically relevant surfaces is currently unavailable, leaving a gap in the ability to survey for potential contamination, and subsequent effective decontamination of such surfaces. The development of such a method could bridge this gap and be implemented as a part of prion surveillance protocols.

**Experimental Design:** An environmental swabbing technique was adapted to clinical and laboratory relevant surfaces, including stainless steel, glass and benchtop. Surfaces were contaminated with a hamster adapted transmissible mink encephalopathy prion strain, HY TME, and disinfected for 10 minutes with either $H_2O$, 70% ethanol or undiluted bleach. These surfaces were then subjected to the adapted swabbing technique, and extracts were investigated with the real time quaking induced conversion assay, RT-QuIC, for seeding activity. For select surfaces, swab extracts were examined for residual infectivity with RT-QuIC and animal bioassay. In addition, stainless steel wires were contaminated and analyzed with RT-QuIC to assess potential remaining surface infectivity.

**Results:** Bleach treatment of HY TME contaminated stainless steel, glass slides and benchtop resulted in a failure to detect RT-QuIC seeding activity from surface swab extracts, while $H_2O$ and 70% ethanol were ineffective disinfectants. Additionally, for glass slide surfaces, hamsters inoculated with bleach treated surface swab extracts failed to develop clinical disease. The RT-QuIC assessment of HY TME contaminated stainless steel wires treated with bleach failed to exhibit seeding activity.

**Conclusions:** The described swabbing methodology coupled with RT-QuIC can be used to successfully recover prions from surfaces, as well as determine disinfection efficacy. The RT-QuIC analysis and animal bioassay of the same swab extracts were congruent. RT-QuIC analysis of surface swab extracts and stainless steel wires resulted in similar results, indicating that disinfected surface swab analysis is an indicator of surface disinfection. Overall, the described method can be used to survey both laboratory and clinical surfaces for potential prion contamination.

DEVELOPMENT OF IN VITRO ORGANOTYPIC SKIN TO STUDY THE LYME DISEASE PATHOGEN

Jaxon J. Kramer, Trenten Theis, Carley M. Conover, Amanda J. Brinkworth (UNMC Omaha, NE)

**Background, Significance, Hypothesis**: Lyme Disease is caused by *Borrelia burgdorferi* and is transmitted via tick-bite. Acute manifestations include rash and flu-like symptoms, while chronic symptoms like arthritis and neurological impairment can remain even after antibiotic treatment. According to the CDC, the U.S. had 62,428 confirmed cases in 2022, with many more suspected. Current models to study *Borrelia* transmission include murine models as well as *ex vivo* human skin. Murine skin differs greatly in structure as well as cellular content, while *ex vivo* human skin is the gold standard, but availability is limited. *This presents a need for a laboratory-generated human skin model that is reproducible and can be used to detect pathogen transmission and dissemination following a tick-bite.*

**Experimental Design**: We have developed a novel tick feeding system that builds upon a well-described *in vitro* 3D-organotypic human skin model that is composed of epidermal (stratified HaCat keratinocytes) and dermal layers (NHDF fibroblasts in collagen). An important aspect of tick feeding is being able to access microcapillaries in the skin. For this reason, we have added endothelial cells (HUVECS) to induce vascularization. HUVECs are grown on collagen beads covered in fibrin then embedded within the dermal collagen layer to act as seeding points for tubules. Additionally, both ticks and *Borrelia* are affected by immune cells during tick feeding, thus we have introduced macrophages derived from $CD14^+$ monocytes (purified from PBMCs) into the dermal layer for up to 20 days. Additionally, we have added MaxGel to the dermal layer which contains a variety of ECM components that are abundant in human skin and can affect skin structure, vascularization, immune cell function and pathogen adhesion. These skin alterations introduced in the organotypic model were compared to *ex vivo* human and porcine skin biopsies to determine biological relevance

To mimic a tick bite in the skin, *B. burgdorferi* were injected directly (5 times per raft) in the presence of tick salivary gland extract and allowed to colonize for 3 days. To mimic re-colonization of the skin following a disseminated infection, *B. burgdorferi* were placed under the transwell holding organotypic skin and left static for 2 days. After 2 days, the rafts were fixed and stained to evaluate *B. burgdorferi* migration into the skin.

**Data and Results**: Using immunofluorescent confocal microscopy, we compared macrophage populations and vascularization between our organotypic skin and biopsies of adult human skin and newborn porcine skin. Monocyte-derived macrophages introduced into organotypic skin lived up to 20 days and displayed markers similar to skin biopsies, such as CD163, but had elongated morphology. This contrasts with *in vitro* cultured macrophages that started rounding up by Day 10 in culture. Addition of collagen beads coated in fibrin and HUVECs into the dermal layer resulted in a few tubules that extended to the base of the dermis but did not have fully formed vessels. The addition of MaxGel increased fibroblast proliferation and keratinocyte differentiation compared to the simple skin model. Also, we have shown that this model is capable of dissemination assays, as *B. burgdorferi* could readily migrate from media below the skin, travel through the dermis and localize in the epidermal layer.

**Conclusion**: We have developed a novel human skin model that can be used for studies of the transmission of tick-borne pathogens, as well as for pathogen dissemination studies within the skin. We expect that our modifications will increase tick feeding and subsequent pathogen transmission. Comparisons between our model and human *ex vivo* and porcine skin have shown that while there are some important similarities between the models, additional work needs to be done to ensure functionality. Further studies will involve addition of other immune cells, such as dendritic cells and Langerhans cells. Since we have demonstrated that *Borrelia* can disseminate into the modified organotypic skin model, we can use it to study host-pathogen interactions and the resulting immune response during tick feeding and pathogen transmission.

**KEEP (PI)CALM AND CARRY ON: MECHANISMS OF GOLGI TRAFFICKING MANIPULATION BY *CHLAMYDIA TRACHOMATIS***

Haley Knowles, Legacy Durham, Elizabeth Rucks (University of Nebraska Medical Center, Omaha, NE)

**Background, Significance, and Hypothesis:** *Chlamydia trachomatis*, a gram-negative obligate intracellular pathogen, is the leading cause of sexually transmitted bacterial infections, disproportionally affecting women of reproductive age. Infections are often asymptomatic and if left untreated, can lead to severe urogenital complications. While antibiotics are effective, they can disrupt healthy vaginal microflora, causing additional complications. Advancing our understanding of chlamydial development and host-pathogen interactions could reveal unique, *Chlamydia*-specific targets for intervention. Within eukaryotic host cells, *C. trachomatis* resides within a vacuole termed the inclusion, where it acquires essential nutrients by manipulating host vesicular trafficking pathways and recruiting host proteins. *C. trachomatis* intercepts Golgi-derived exocytic vesicles to obtain essential sphingomyelin and cholesterol, and acquires transferrin via the endosomal recycling pathway. The eukaryotic protein PICALM, which supports clathrin-mediated endocytosis and regulates cholesterol and iron homeostasis, may link these two independent trafficking pathways. Our lab has shown that PICALM localizes to the inclusion and may regulate nutrient acquisition, but whether and/or how *Chlamydia* modulates PICALM and these pathways remains unclear. PICALM may act as a gatekeeper to modulate chlamydial access to nutrients from endocytic and exocytic pathways. Therefore, we hypothesize that *C. trachomatis* alters intra-Golgi trafficking kinetics, mimicking or co-opting PICALM to manipulate Golgi function and support chlamydial development.

**Experimental Design, Data, and Results:** To test this hypothesis, we utilized HeLaM cells expressing a fluorescent secretory reporter (ss-eGFP-FKBP$^{F36M}$). This reporter, containing four dimerization domains, forms large multimers in the ER that prevent transport. Treatment with 1 μM rapamycin (a D/D solubilizer) disassembled these multimers, enabling reporter secretion along the vesicular trafficking pathway: ER → Golgi → trans-Golgi network → plasma membrane. Cells were fixed and stained for indirect immunofluorescence imaging via confocal microscopy. Using Pearson's coefficient to analyze GFP colocalization with the Golgi, we quantified changes in secretory trafficking kinetics in uninfected and C. trachomatis serovar L2-infected cells throughout chlamydial development. For PICALM knockdown studies, HeLaM cells were infected or left uninfected, then transfected with either NT siRNA or PICALM siRNA.

At 8 hours post-infection (hpi), intra-Golgi trafficking kinetics were similar in uninfected and chlamydial-infected cells. At 16 hpi, we observed an accelerated rate of cargo transit from Golgi to trans-Golgi in chlamydial-infected cells. This effect seems to diminish by 24 hpi, but we observed significantly less reporter localizing within the Golgi, suggesting altered cargo trafficking or production. At 40 hpi, secretory trafficking slowed significantly in infected cells, with cargo accumulating in the Golgi and trans-Golgi instead of progressing to the plasma membrane as in uninfected cells. PICALM knockdown via siRNA at 24 hpi slowed ER-to-Golgi trafficking in uninfected cells but had little effect in infected cells, suggesting chlamydial infection may nullify PICALM knockdown effects. However, increased retention of secretory cargo within the Golgi and trans-Golgi was observed in both infected and uninfected cells during PICALM knockdown.

**Conclusions:** *Chlamydia* alters intra-Golgi trafficking kinetics throughout its development, with early chlamydial infection mimicking intra-Golgi trafficking kinetics in uninfected cells while mid-to-late infections show much slower intra-Golgi trafficking kinetics. PICALM knockdown increases the retention of secretory cargo within the Golgi in both infected and uninfected cells, suggesting a novel role for PICALM in Golgi trafficking. Future studies will attempt to further clarify PICALM's functions via an APEX2 proximity labeling system to map the Golgi interactome. This will reveal alterations in intra-Golgi protein interactions and identify novel vesicular trafficking proteins influenced by chlamydial infection and/or PICALM. Overall, this project will clarify if there is a function for PICALM in Golgi trafficking and will improve our understanding of how *Chlamydia* may exploit host machinery, particularly the Golgi, to support its development and pathogenesis.

# IDENTIFICATION OF METHYLATION ON THE CERE OF *NAEGLERIA SPECIES*

**Nik Johnson[1], Kristen Drescher[1]**

[1]Creighton University School of Medicine, Department of Medical Microbiology and Immunology, Omaha, NE

**Background**. The ribosomal DNA (rDNA) of *Naegleria* is encoded on a closed circular extrachromosomal element (CERE) in the nucleolus of the trophozoite. It is estimated that one trophozoite contains ~4,000 CERE copies. Most of the CERE sequence is comprised of the non-ribosomal sequence (NRS) which is believed to contain a single origin of replication. Based on the limited number of complete CERE sequences available, the NRS regions have little sequence conservation between species, while the rDNA cistrons are highly conserved. It is postulated that the NRS regions are involved in regulation of the ribosomal genes.

**Significance of Problem**. Regulation of gene transcription by the CERE of *Naegleria* is largely unexplored, but understanding how ribosome expression is regulated may provide insight into how to target treatments against *Naegleria fowleri* infections in humans, which are greater than 97% fatal. One general mechanism of gene regulation in eukaryotes is via DNA methylation. DNA methyltransferase enzymes have been described in *Naegleria*, thus it appears that *Naegleria* could use methylation as a form of gene regulation.

**Hypothesis, Problem, or Question**. We hypothesized that methylation of the CERE of *Naegleria gruberi, Naegleria jadini, Naegleria australiensis,* and *Naegleria pringsheimi* occurs.

**Experimental Design**. Trophozoites were harvested from four species of *Naegleria* and CERE isolated. Isolated CERE were sequenced using Oxford Nanopore Technologies, the resulting data was then analyzed using dorado to determine the methylation status of cytosine and adenine.

**Results/Data**. Methylation was present in CERE from all *Naegleria* species examined. Methylation was found at throughout the CERE, with little to no analogous locations of methylation between the four species investigated. No position on any of the species had more than 2% of the reads identifying cytosine methylation. However, all CERE had high levels of adenine methylation slightly upstream of the 18s rDNA sequence. All four CERE had between 25-45% of their adenines methylated in this region.

**Conclusions.** Based on the results, methylation may be a form rDNA regulation in *Naegleria* trophozoites. The level of methylation identified in these studies is on the lower end of what has been reported in other eukaryotes. However, methylation may not be the only form of gene regulation used by the *Naegleria*; other forms of regulation could include transcriptional and post-transcriptional regulation or the formation of G4-quadruplex structures. The large number of predicted CERE per trophozoite coupled with the low percentage of overall methylation occurring, may indicate that there are multiple regulatory mechanisms used by the *Naegleria*. The presence of cytosine methylation in general however points towards it being involved in gene regulation. The abundance of adenine methylation upstream of the 18s rDNA subunit could provide insight into the location of the transcriptional start site of the rDNA. Further studies will be performed to investigate whether the patterns of methylation observed in the trophozoite (replicating life stage) differs from those observed in the cyst (hibernation stage).

# THE SPX REDOX SWITCH CONTROLS CYSTINE UPTAKE AND TOXICITY IN *STAPHYLOCOCCUS AUREUS* UNDER DISULFIDE STRESS

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**Background, Significance, Hypothesis:** The Spx protein is an essential redox-sensitive transcriptional regulator that responds to disulfide stress and maintains thiol homeostasis in the pathogen *Staphylococcus aureus*. The formation of an oxidized disulfide in the Spx redox switch enables it to facilitate expression of various genes involved in thiol homeostasis. In *S. aureus,* Spx is essential for growth. However, the role of its redox switch has not been explored.
*S. aureus* poses a severe threat to public health, and the rise of antibiotic-resistant strains highlights the need to develop new therapeutics. Given its central role in maintaining bacterial thiol homeostasis, Spx function could be vital for *S. aureus* when responding to immune cell-mediated oxidative stress. Additionally, thiol redox homeostasis is essential for bacterial survival, so understanding the mechanisms involved could lead to the identification of novel antimicrobial targets. We seek to identify the functional significance of the Spx redox switch in *S. aureus* stress response.

**Experimental Design:** We successfully created a targeted Cys→Ala mutation in the *S. aureus spx* redox switch, preventing the switch from responding to oxidative stress. The resulting *spx*$^{C10A}$ mutant was challenged with diamide, a treatment known to oxidize intracellular thiols and grown for 24 hours in Tryptic Soy Broth, with growth quantified via $OD_{600}$ every 0.5 hours. The impact of *spx*$^{C10A}$ mutation on the maintenance of thiol homeostasis was determined using a redox-sensitive GFP. RNAseq was performed to determine the transcriptional regulon of the Spx redox switch in the presence and absence of diamide. Ellman's reagent was used to measure total intracellular low molecular weight thiol levels after diamide treatment. Total cellular iron was quantified using inductively coupled plasma mass spectrometry (ICP-MS) and intracellular free iron was measured using sensitivity to the antibiotic streptonigrin.

**Results and Conclusion:** We demonstrate that the impaired growth of the *spx*$^{C10A}$ mutant does not arise from its inability to maintain intracellular thiol homeostasis following diamide challenge. Rather, the *spx*$^{C10A}$ mutant efficiently adapts and counters thiol oxidation through increased cystine uptake. But paradoxically, our findings reveal that increased cystine uptake itself is toxic to the growth of the *spx*$^{C10A}$ mutant during disulfide stress. Inactivating the major cystine transporters in *S. aureus* prevented the *spx*$^{C10A}$ mutant from adapting to disulfide stress but unexpectedly restored its growth. We determined the mechanism of this cysteine toxicity is chelation of free intracellular iron, causing a depletion in iron available for enzymatic reactions. **These findings indicate that *S. aureus* may utilize redox signaling through Spx to limit toxicity from cystine uptake during disulfide stress.**

# O-09

INVESTIGATING THE ROLE OF *S. AUREUS* CITRATE METABOLISM DURING PROSTHETIC JOINT INFECTION

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**Background:** *Staphylococcus aureus* (*S. aureus*) is a common member of the human microbial flora, with ~30% of the population colonized. However, it is also an opportunistic pathogen and the leading cause of medical device-associated biofilm infections, such as prosthetic joint infection (PJI). Biofilm formation evolves as bacteria multiply and deposit a complex extracellular matrix that creates a heterogeneous population with unique metabolic properties. *S. aureus* is metabolically flexible, allowing it to colonize various niches and establish chronic infection. Several bacterial tricarboxylic acid cycle (TCA) intermediates are important for biofilm development. Our laboratory has characterized an *in vivo* mouse model of PJI and identified macrophages (Mφs), anti-inflammatory granulocytic myeloid-derived suppressor cells (G-MDSCs), and neutrophils (PMNs) as the predominant leukocyte infiltrates, all of which are also present in human PJI. This study utilized a novel bacterial single-cell sequencing platform to identify changes in the transcriptional profile of *S. aureus* biofilms when challenged with Mφs, G-MDSCs, and PMNs, uncovering increases in genes encoding TCA cycle enzymes. Further studies interrogated infectivity of *S. aureus* mutants lacking citrate synthase (Δ*gltA*), aconitase (Δ*acnA*), and isocitrate dehydrogenase (Δ*icd*) in a mouse model of PJI. Additionally, intracellular survival of these metabolic mutants in macrophages was examined. Future studies will investigate how adaptations in *S. aureus* TCA cycle metabolism aid in shaping the immune response during PJI.

**Significance of Problem:** PJI is a complication following arthroplasty that occurs in ~1-5% of patients, with more complex procedures associated with increased infection risk. *S. aureus* is a leading cause of PJI with ~50% of cases resulting from methicillin-resistant *S. aureus*. Treatment for PJI generally consists of revision, followed by a second surgery for prosthesis replacement. Understanding how initial *S. aureus*-leukocyte metabolic interactions promote biofilm formation could aid in the development of early treatment interventions.

**Question:** What is the role of *S. aureus* citrate metabolism in adapting to leukocyte pressure and biofilm formation during PJI?

**Experimental Design and Results:** Using our novel bacterial single-cell sequencing platform we identified increased transcription of citrate synthase (*gltA*), aconitase (*acnA*), and isocitrate dehydrogenase (*icd*) in mature biofilm challenged with Mφs, G-MDSCs, and PMNs. These findings were supported by liquid chromatography-high resolution mass spectrometry (LC-HRMS), which identified elevated TCA cycle metabolites in *S. aureus* biofilm co-cultured with G-MDSCs compared to biofilm alone (Unpaired t-test). Next, to determine if the enhanced *gltA* expression occurred in discrete biofilm microdomains or was homogeneous, a fluorescent reporter construct driven by the native *gltA* promoter (*pgltA*-GFP) was examined by confocal microscopy. Augmented *gltA* transcription occurred throughout the biofilm following Mφ co-culture. In contrast, minimal *pgltA*-GFP expression was detected in biofilm alone that was restricted to distinct domains. To investigate the functional role of citrate metabolism during PJI, animals were infected with citrate metabolism mutants vs. wild type (WT) *S. aureus*. Bacterial burdens were significantly decreased with the *gltA* mutant (Δ*gltA*) compared to WT *S. aureus* in the knee and implant at day 7 post-infection (one-way ANOVA). However, this effect was transient since differences were no longer evident at day 14. Furthermore, Δ*acnA* and Δ*icd* had similar bacterial burdens to WT infected mice at both time points (one-way ANOVA), collectively suggesting that *S. aureus* can adapt to limiting TCA cycle metabolites. Since citrate synthase is important during early PJI, we next assessed if it was critical for *S. aureus* intracellular survival in Mφs. Mφs were exposed to live *S. aureus* at a multiplicity of infection (MOI) of 10:1 (bacteria:leukocyte) for 1 h before treatment with high dose gentamicin to kill remaining extracellular bacteria. Mφs were lysed and bacteria enumerated at 0, 2, 4, and 24 h post-infection to evaluate the kinetics of bacterial survival. Surprisingly, at early time-points (2 and 4 h) citrate metabolic mutants had higher survival compared to WT; however, this was diminished at 24 h post-infection (two-way ANOVA), reminiscent of the transient *in vivo* phenotype of Δ*gltA* during PJI.

**Conclusion:** These data indicate that under immune cell pressure, *S. aureus* biofilm increases TCA cycle activity and production of related metabolites, as measured by LC-HRMS. Furthermore, transcription of enzymes involved in citrate metabolism are increased in biofilm following immune cell exposure. Citrate synthase (*gltA*) promotes early PJI; however, during chronic infection, *S. aureus* seems able to compensate for the loss of citrate synthase. Interestingly, TCA cycle deficiency seems to be beneficial for acute bacterial intracellular survival. However, the mechanism for this remains undefined. Future studies will identify how *S. aureus* compensates for the loss of TCA cycle activity, which may involve carbon shunting via nitrogen synthesis, as carbon can re-enter the TCA cycle through the generation of α-ketoglutarate from glutamate, which could offset TCA insufficiency.