**55th Annual Midwest Student Biomedical Research Forum**

Saturday, March 2, 2024

# ROOM 3040

10:15 a.m. **O-02** THE ROLE OF THE ION TRANSPORTER CLC7 DURING INFECTION BY *COXIELLA BURNETII*
*Presenter: Sushmita Adhikari, UNMC*

10:30 a.m. **O-09** LEUKOCYTE HETEROGENEITY IN RESPONSE TO *S. AUREUS*
*Presenter: Nichole Brandquist, UNMC*

10:45 a.m. **O-14** MULTIPLEX ASSAY REVELS DIVERSE ANTI-INFLUENZA IgG BINDING SPECIFICITIES TO HEMAGGLUTININ PROTEINS
*Presenter: Del Dsouza, UNMC*

11:00 a.m. **O-21** MULTIPLE EXPOSURE HISTORY RESULTS IN ANTIBODIES WHICH RECOGNIZE PROTEINS OF NON-EMERGED SARS-COV-2 VARIANTS
*Presenter: Dylan George, UNMC*

11:15 a.m. **O-49** DEVELOPMENT OF A TRANSFECTION METHOD FOR NAEGLERIA SPP. USING A MOLECULAR CLONE OF N. GRUBERI
*Presenter: Brian Nguyen, Creighton University*

11:30 a.m. **O-73** IDENTIFICATION OF CELLULAR FACTORS INFLUENCING HIV-1 SUSCEPTIBILITY
*Presenter: Kayla Weldon, Creighton University*

11:45 a.m. Break

# THE ROLE OF THE ION TRANSPORTER CLC7 DURING INFECTION BY *COXIELLA BURNETII*

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*Coxiella burnetii* is an intracellular gram-negative bacterium that is the causative agent of Q fever. *Coxiella* replication occurs in a phagolysosome-like vacuole termed the *Coxiella* Containing Vacuole (CCV), an acidic compartment which is essential for *Coxiella* replication. The bacteria actively regulates CCV pH in a range conducive for bacterial metabolic activity while simultaneously decreasing the activity of host microbe-killing proteases. An ideal pH is required for bacterial replication; a mature CCV pH is ~5.2, whereas a pH of 4.5 will kill the bacterium. The CCV also has other peculiar properties, such as a sterol-rich membrane. However, elevated CCV membrane cholesterol leads to rapid CCV acidification, suggesting ion channels may be affected. The lysosomal chloride antiporter CLC7 plays a critical role in regulating lysosomal pH. Intriguingly, it contains a cholesterol-binding domain, although the role of cholesterol in regulating CLC7 activity is unknown. **Significance of Problem.** Q fever can lead to culture-negative endocarditis, a chronic disease with a high mortality rate. There is no licensed Q fever vaccine in the United States, and treatment involves prolonged antibiotic regimens. **Hypothesis**. The purpose of this research is to determine whether CLC7 is critical to maintain CCV pH during infection. **Experimental Design/ Results**. The research hypothesis explores whether CLC7 is essential for bacterial metabolism and CCV pH maintenance during infection. Ectopic expression of mCherry-tagged CLCN7 in *Coxiella*-infected cells suggests that CLC7 localizes to the CCV, and therefore may play a role in regulating CCV pH. *Coxiella* infection of wildtype and CLC7 knockout cells revealed a significant defect in CCV size in the absence of CLC7. Surprisingly, a colony-forming unit (CFU) bacterial growth assay indicates improved *Coxiella* growth in CLCN7 KO cells, contradicting the correlation between CCV size and bacterial load. **Conclusions**. Our data suggests that CLC7 plays a role in CCV formation and bacterial growth during *Coxiella* infection. The study underscores the need for a deeper understanding of CLC7's contributions to *Coxiella* pathogenesis, as this knowledge could pave the way for Q fever prevention and less intensive treatment strategies.

# LEUKOCYTE HETEROGENEITY IN RESPONSE TO *S. AUREUS*

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**Background:** *S. aureus* is a leading cause of medical device-associated biofilm infections. Biofilm formation evolves as bacteria multiply and produce a complex extracellular matrix that creates a heterogeneous population with unique metabolic properties. Our previous work has focused on how mature biofilm evades immune-mediated clearance; however, the role of leukocytes during early biofilm development remains largely uncharacterized. Preliminary *in vitro* studies have demonstrated that macrophages are extremely susceptible to mature *S. aureus* biofilm. Additionally, mitochondrial ROS production (mtROS) may be linked to macrophage death in the presence of biofilm. In our mouse model of *S. aureus* prosthetic joint infection (PJI), anti-inflammatory granulocytic myeloid-derived suppressor cells (G-MDSC) represent the main leukocyte infiltrate followed by polymorphonuclear leukocytes (PMN), and macrophages. However, the differential sensitivity of each leukocyte population to *S. aureus* and how this may influence biofilm development remains unknown. This study examined macrophage, G-MDSC, and PMN viability when challenged with both planktonic bacteria and mature biofilm. Additionally, MitoSOX and ApoTracker were used to measure early signs of cellular stress and apoptosis, respectively with three different *S. aureus* mutant strains (*agr*, *psm/δ toxin*, and *hla/lukAB*) to investigate the role of these virulence factors in leukocyte responses. These findings will advance our understanding on how early *S. aureus*-leukocyte interactions promote biofilm formation.

**Significance of Problem:** Prosthetic joint infection (PJI) is a complication from arthroplasty that occurs in approximately 1-2% of patients, with a ~20-30% infection rate for more complex procedures. *S. aureus* is the leading causative pathogen for PJI with ~50% of cases caused by methicillin-resistant *S. aureus* (MRSA). Treatment for PJI generally requires removal of the infected implant, followed by a second surgery for prosthesis replacement. Understanding early *S. aureus*-leukocyte interactions and how they promote biofilm formation could aid in the development of early treatment interventions.

**Question:** What are the differences in leukocyte viability, mtROS production, and apoptosis in response to planktonic *S. aureus* and mature biofilm?

**Results:** To investigate the effect of planktonic *S. aureus*, leukocytes were challenged with bacteria at a multiplicity of infection (MOI) of 10:1 (bacteria:leukocyte) for 30 min, 2 h, and 6 h. To investigate biofilm sensitivity, leukocytes were exposed to a mature biofilm for 15 min, 30 min, 2 h, and 6 h. Following co-culture, leukocytes were stained with a CD45 antibody, Zombie dye (viability), MitoSOX Red (mtROS), and ApoTracker Green (apoptosis) and analyzed via flow cytometry. Macrophages exhibited drastic differences in their response to planktonic *S. aureus* vs. biofilm, with ~70% of macrophages still viable at 2 h with planktonic bacteria, whereas less than 50% were viable after a 15 min co-culture with biofilm. In general, these changes coincided with an increase in mtROS and apoptosis markers, except under conditions where high cell death was observed. In contrast, G-MDSCs were highly sensitive to planktonic bacteria but less affected by biofilm, with little to no viable G-MDSCs in planktonic co-cultures at 6 h, while those exposed to mature biofilm remained ~20% viable. PMNs were the most recalcitrant to either planktonic or mature biofilm when compared to the other leukocyte populations, with ~20% and 60% viable cells at 6 h, respectively. Again, increases in mtROS and apoptosis markers were observed in the granulocytes, except in instances of high cell death. None of the *S. aureus* mutants examined were able to restore viability in any of the leukocyte populations, suggesting virulence factor redundancy.

**Conclusion:** These results establish that macrophages, G-MDSCs, and PMNs respond differently to planktonic *S. aureus* and mature biofilm. It is known that macrophages challenged with planktonic *S. aureus* are more pro-inflammatory, which we show is associated with prolonged viability. This was the opposite for G-MDSCs, which demonstrated poor viability with planktonic *S. aureus* compared to biofilm, supporting previous findings that G-MDSCs play a role in skewing macrophages to an anti-inflammatory state in the presence of mature biofilm. To better understand the early mechanisms by which *S. aureus* exerts its cytotoxic effects and skews the biofilm niche towards an anti-inflammatory milieu, future studies will aim to identify *S. aureus* virulence factors at these acute time points and their role in both leukocyte cytotoxicity and biofilm development.

# MULTIPLEX ASSAY REVELS DIVERSE ANTI-INFLUENZA IgG BINDING SPECFICITIES TO HEMAGGLUTININ PROTEINS

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**Background:** Influenza viruses infect millions globally and cause significant burden to worldwide heathcare infrastructure. Understanding the humoral immune responses to influenza viruses poses challenges due to antigenic imprinting and multiple exposures to infections and vaccines.

**Significance of Problem:** Complexities involvilng exposures to infection and vaccines complicates the assessment of individuals' immune competence for risk evaluation and developing a universal influenza vaccine.

**Hypothesis:** Our in-lab influenza antibody assay, accounting for interindividual variability, will effectively address existing challenges, as demonstrated in a proof-of-concept pilot study.

**Experimental Design:** We comprehensively searched indexed and grey literature, surveillance, and sequence databases including Global Initiatives on Sharing All Influenza Data (GISAID) to identify predominant influenza viruses from 1968-2023. We assessed relationships between birth cohorts, virus exposures, and antigenic changes in immunodominant hemagglutinin epitopes. Archetypal strains were selected based on these criteria. Amino acid alignment and distance measurements were performed using the Jukes-Cantor model. Commercial protein constructs were used to construct a multiplex, bead-based immunoassay on the Luminex xMAP INTELLIFLEX System. Plasma specimens were analyzed from selected participants in the Clinical Characterization Protocol for Severe Emerging Infections study (CCPSEI; UNMC IRB 146-20-FB) with variable influenza virus and vaccine exposure histories. Descriptive analyses were performed using GraphPad Prism version 10.1.0.

**Results:** Four distinct birth cohorts were identified. Amino acid alignment of immunodominant epitopes guided the inclusion of four H3N2 and three H1N1 antigenically distinct hemagglutinin proteins in the assay, which were successfully coupled and verified with control antibodies. In the pilot study with nine CCPSEI participants (born between 1946 and 1997), differences in antibody binding were observed between older and more contemporary strains, particularly against H3 in participants born in years consistent with H3N2 primary exposure. Inter-individual antibody binding variability to H1 and H3 was evident across incorporated strains.

**Conclusions:** Birth year may influence antibody binding to antigenically distinct influenza viruses, even in individuals with repeated influenza vaccinations. Further investigations with larger populations are essential to comprehensively characterize the extent and factors influencing inter-individual antibody response variability.

# MULTIPLE EXPOSURE HISTORY RESULTS IN ANTIBODIES WHICH RECOGNIZE PROTEINS OF NON-EMERGED SARS-COV-2 VARIANTS

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**Background** SARS-CoV-2 continues to evolve, and alongside it so do our efforts to protect communities against severe disease. These efforts have taken the form of various public health measures and new vaccine formulations. Recently, focus has been placed on vaccines targeting variant specific antigens in the hope of providing superior immune protection by avoiding neutralizing antibody evasion.

**Significance of Problem** Understanding the role of antibody-mediated immunity at the population level is valuable to inform public health decisions relating to SARS-CoV-2. This is complicated by the multi-year COVID-19 pandemic where the population has had complex exposure history to multiple viral variants and vaccine formulations. The influence of previous exposures and pre-existing immunity to SARS-CoV-2 and its influence on the immune response to subsequent exposures is not well understood.

**Question** How do previous SARS-CoV-2 exposures, whether by infection or vaccine, impact antibody specificity to variant-specific antigens in the setting of serial exposures?

**Experimental Design** Using the xMAP IntelliFlex®, we developed a multiplex immunoassay (HumorX) that simultaneously measures antibodies targeting the SARS-CoV-2 nucleoprotein (N) as well as multiple variants of the receptor binding domain (RBD) including those from canonical, Omicron BA.5, and XBB.1.5 strains. Recombinant proteins were coupled in house to MagPlex microspheres. Assay performance was characterized using plasma samples collected before and during the COVID-19 pandemic by the Nebraska BioBank (RRID:SCR_021024). This study utilized specimens obtained from the Clinical Characterization Protocol for Severe Emerging Infections (CCPSEI), a year-long prospective observational cohort study (IRB No. 0146-20-FB). Specimens were compared based on time since last exposure as well as by breaking the study period into a pre- and post-Omicron time frame.

**Results** HumorX demonstrated high clinical specificity (99.3% anti-RBD/N IgG, 99.6% anti-RBD/N IgM) and high clinical sensitivity (100% positivity 15-90 days post-PCR confirmed COVID-19). We observed that multiple exposures, even to non-Omicron strains or vaccines, lead to an increase in antibody binding to Omicron and XBB RBD antigens. Despite this increased binding, the response to the canonical RBD remains dominant.

**Conclusions** Using a novel multiplex immunoassay, we detect antibody responses with broader reactivity to variant SARS-CoV-2 RBD proteins following serial infection and/or vaccine exposures. This test has been made possible thanks to the use of xMAP technology combined with in-lab developed protocols. There is an ongoing discussion about the role that immune imprinting, a phenomenon typically associated with influenza, plays regarding the SARS-CoV-2 antibody repertoire following exposure. Further research is warranted to determine both the affinity of these variant induced antibodies as well as the impact they play in host-protection.

# DEVELOPMENT OF A TRANSFECTION METHOD FOR NAEGLERIA SPP. USING A MOLECULAR CLONE OF N. GRUBERI

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## Background

Naegleria are eukaryotic single-cell amoebas that are ubiquitous in freshwater environments and can exist as trophozoites, flagellates, or cysts depending on their environment and available resources. Feeding and replication occur during the trophozoite phase, motility during the flagellated phase, and dormancy during the cyst phase when resources are scarce. In the Naegleria genus, only *N. fowleri* (also known as the 'brain eating amoeba') is pathogenic to humans while the non-pathogenic, *N. gruberi*, is often used to study the biology of Naegleria species. Naegleria spp. encode all of their ribosomal DNA (rDNA) genes on closed circular extrachromosomal rDNA elements (CERE) located in the nucleolus. These CERE have their own origin of replication (*ori*) and presumably replicate independently and autonomously from the chromosomal DNA. A single Naegleria trophozoite contains 3000-5000 copies of the CERE yet little is known about how the CERE functions and replicates in the amoeba. Herein, we report the development of a tool that will enable us to study CERE replication in Naegleria and follow the replication dynamics across different life stages.

## Hypothesis

We hypothesize that the CERE of *N. gruberi* contain a conserved *ori* that allows the transfection of foreign DNA into *N. gruberi* trophozoites that is maintained throughout several passages of trophozoites and through encystment and excystment of Naegleria cells.

## Experimental Design

A molecular clone of the *N. gruberi* (pGRUB) was obtained that contains the entirety of the *N. gruberi* CERE ligated to a prokaryotic pGEM vector. Using ViaFect Transfection Reagent (Promega), pGRUB was transfected into *N. gruberi* trophozoites. After one hour, the cells were treated with DNase to ensure that there were no residual pGRUB. Transfected cells were cultured for seven passages; at each passage, half of the cells were utilized for DNA isolation and the other half for further cell culture. After seven passages, the cells were then cultured with encystment buffer to induce cyst formation of the *N. gruberi* trophozoites. Cysts were harvested at 48 hours for DNA isolation and the remaining cells were excysted back to trophozoites. *N. gruberi* cells were left to excyst and replicate in growth media for 72 hours before a final harvest for DNA isolation. Control cultures of trophozoites were transfected with a pGEM, that lacks an eukaryotic origin of replication. PCR using primers specific for either pGRUB or pGEM construct was performed on DNA isolated at each passage. These primers differentiate between the native CERE, the transfected pGRUB, and the transfected pGEM control

## Results

PCR followed by gel electrophoresis was performed on DNA isolated from each passage of the transfected trophozoites. Trophozoites that were transfected with pGRUB yielded a positive signal at 656bp when PCR was performed using pGRUB-specific primers. pGRUB-transfected *N. gruberi* maintained signal through all passages examined and both the encystment and excystment processes. The transfection of the prokaryotic pGEM vector produced signal within the first two passages, but the subsequent passages, as well as the encystment and excystment, did not produce any detectable PCR product

## Conclusion

Our results show the successful transfection of a foreign vector construct, pGRUB, into *N. gruberi,* as well as propagation of this molecular clone across several passages. The construct was maintained through differing morphological phases indicated by the results from the encystment and excystment processes. Conversely, our negative control (pGEM) did not produce any detectable signal beyond the first two passages. This indicates a transient pGEM presence that cannot propagate without the existence of a eukaryotic *ori*. These studies are the first to demonstrate that self-replicating Naegleria CERE can be transfected into trophozoites, and that the CERE are maintained throughout the encystment and excystment processes. Together, these data provide the basis for further studies defining the mechanism of CERE replication in Naegleria.

IDENTIFICATION OF CELLULAR FACTORS INFLUENCING HIV-1 SUSEPTIBILITY

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## 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. HIV infection remains 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

Cells with altered susceptibility to HIV infection will have distinct changes in the expression or function of cellular factors and pathways. Identification of these will lead to novel strategies to inhibit HIV replication.

## Experimental Design

Numerous Jurkat T cell lines treated with CRISPR-Cas9 were cloned by limiting dilution, expanded, then assayed for HIV-1 susceptibility. Susceptibility was measured through single-round infection assays with an HIV-1 virus containing a luciferase reporter gene (HIV-Luc). RNA was isolated from seven cell lines with the most differential susceptibility. Total RNA sequencing was performed using Illumina’s TrueSeq Total RNA – Gold prep kit and run on a p2 / 200 cycle kit on the NextSeq™ 2000 sequencing system. RNA sequencing data was analyzed using Illumina’s onboard DRAGEN workflow and Tuxedo tools suite. Differentially expressed genes (adjusted p value of <0.001) were ranked by a $\log_2$ change from parental Jurkat E6-1 cell line. The differential expression of the top candidate genes 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. The protein expression of individual factors was determined by quantitative western blot using GAPDH as a control. Over-expression of candidate factors was achieved by pre-transfection of a plasmid expression vector 24 hours prior to infection with HIV-Luc reporter virus. Over-expression of candidate proteins was confirmed by immunoblot. All statistics were performed using GRAPHPAD PRISM Software version 10.1.

## Results/Data

Susceptibility screening identified 7 Jurkat cell lines with substantial differences in susceptibility (3 high and 4 low) relative to the Jurkat E6-1 parental cell line. RNA sequencing produced a high yield of differentially expressed genes, with 6875 total factors differentially expressed in one or more cell line relative to the parental line. Candidates were ranked by change in expression relative to the parental line as defined by an adjusted p value of <0.001 and $\log_2$ change of >1.0. The 44 most differentially expressed, relative to parental line, were validated by qRT-PCR. Genes with poor amplification and melt curves were eliminated from subsequent experiments, and not evaluated further. In total, the differential expression of 32 of the 44 genes were validated by qRT-PCR. Of those 32 factors, the top 5 candidates were assessed for protein expression by immunoblot. Among those, Sorting Nexin 20 (SNX20) was found to have increased expression in the 3 cell lines with high susceptibility. Elevated levels of SNX20 through plasmid over-expression correlated to a proportional increase in HIV-1 infection susceptibility.

## Conclusions

Cell lines with differential susceptibility to HIV-1 infection display distinct patterns of gene expression. The expression of SNX20 was discovered to correlate with susceptibility to HIV-1 infection. Ectopic over-expression of SNX20 lead to an increase in infection. These data suggest SNX20 is a positive regulator of HIV-1 infection.