56th ANNUAL MIDWEST STUDENT BIOMEDICAL RESEARCH FORUM

Saturday, March 8, 2025

ROOM 3042

8:00 a.m.	O-70	REPEATED RADIOFREQUENCY ABLATION PROMOTES ANTITUMOR IMMUNITY VIA NEUTROPHIL-MEDIATED CCL3-CCR4 AXIS IN PANCREATIC CANCER Presenter: Lincoln Strickland
8:15 a.m.	O-05	IRON DEPENDENT TRANSCRIPTIONAL REGULATION LINKS PATHOGEN RECOGNITION TO NUTRITIONAL IMMUNITY Presenter: Monisha Alla
8:30 a.m.	O-51	From Fighters to Suppressors: Neutrophil Reprogramming into Granulocytic Myeloid-derived suppressor cells (G-MDSCs) during Staphylococcus aureus Biofilm infection Presenter: Adedayo Ogunware
8:45 a.m.	O-39	INTEGRATED STRESS RESPONSE DRIVES IMMUNE DYNAMICS AND BIOFILM PERSISTENCE DURING S. AUREUS CRANIOTOMY INFECTION Presenter: Artha Govind Lotlikar
9:00 a.m.	O-71	SERINE PROTEASES MODULATE NEUTROPHIL IMMUNOSUPPRESSION AND SURVIVAL. Presenter: Reegan Sturgeon
9:15 a.m.	O-67	TRIPLE NEGATIVE BREAST CANCER CELLS ACQUIRE LYMPHOCYTE PROTEINS AND GENOMIC DNA DURING TROGOCYTOSIS WITH T CELLS Presenter: Anutr Sivakoses
9:30 a.m.	O-42	RENAL CANCER CELLS ACQUIRE IMMUNE SURFACE PROTEIN THROUGH TROGOCYTOSIS AND HORIZONTAL GENE TRANSFER Presenter: Haley Marcarian
9:45 a.m.		**BREAK**

REPEATED RADIOFREQUENCY ABLATION PROMOTES ANTITUMOR IMMUNITY VIA NEUTROPHIL-MEDIATED CCL3-CCR4 AXIS IN PANCREATIC CANCER

Lincoln N. Strickland, Nicolette R. Mardik, Casey J. Van Kirk, Nirav Thosani, Jennifer M. Bailey-Lundberg (UNMC, Omaha, NE; McGovern Medical School, Houston, TX)

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is characterized by a hypoxic and immune suppressive tumor microenvironment (TME) and few treatments have resulted in improvements in clinical outcomes. We have previously demonstrated the safety and efficacy of an endoscopic ultrasound-guided, radiofrequency ablation (RFA)-based local ablative therapy, which is currently being tested in clinical trials for locally advanced and metastatic PDAC. In preclinical studies, we showed that a single RFA session induces tumor necrosis and enhances immune cell infiltration, including neutrophils and CD8+ T cells, in both the treated and contralateral tumors (abscopal effect).

Hypothesis: Based on these studies, we *hypothesized* that repeated RFA sessions would further restrain tumor growth and promote increased immune cell infiltration, particularly neutrophils, compared to a single RFA session.

Methods: To test our hypothesis, we performed three repeated RFA sessions in a tumor-bearing *Kras*^{G12D}*; Trp53*^{R172H}*/+; Pdx1:Cre* (*KPC*) syngeneic model, followed by single-cell RNA-sequencing (scRNA-seq), histological analysis, and cytokine profiling.

Results: We found that repeated RFA significantly reduced tumor growth rates and enhanced necrosis in both the treated and contralateral tumors compared to a single RFA session. Repeated RFA also remodeled the immune microenvironment, notably increasing the presence of an N1-like neutrophil cluster. scRNA-seq revealed that these neutrophils had elevated expression of *Ccl3*, a proinflammatory chemokine that binds to CCR4, and *H2-t23*, which encodes for MHC Class I molecules. Additionally, T cell clusters showed increased expression of *Ccr4*.

Discussion: These findings suggest that repeated RFA enhances immune signaling through the CCL3-CCR4 axis, with N1-like neutrophils potentially priming a T cell-mediated antitumor response. Our findings highlight the potential of repeated RFA sessions to not only enhance tumor necrosis and growth suppression but also to stimulate a neutrophil-dependent proinflammatory immune response that may improve the efficacy of PDAC immunotherapy.

IRON DEPENDENT TRANSCRIPTIONAL REGULATION LINKS PATHOGEN RECOGNITION TO NUTRITIONAL IMMUNITY

Monisha Alla, Nick Pokorzynski, Jeonghoon Lee, Abigail Swoboda, Scot Ouellette, Rey Carabeo (UNMC Omaha, NE)

Background, Significance, Hypothesis: *C. trachomatis (Ctr)* is an obligate intracellular pathogen that employs several strategies to evade host immune responses while replicating within permissive cell types, such as epithelial cells. Being compartmentalized within a protective membrane-bound vacuole known as inclusion to sequester chlamydial pathogen-associated molecular patterns (PAMPs), as well as modification of its lipopolysaccharide (LPS) prevent detection through various pathogen recognition receptors (PRRs). A new paradigm of pathogen recognition implicates pathogen-induced errors (infection infidelity) as responsible for activating PRRs. For example, release of intracellular pathogen-associated molecular patterns (PAMPs), such as nucleic acids, cyclic dinucleotides, peptidoglycan (PG) components, etc. would only occur when bacterial membrane integrity is compromised because of dysregulated biochemical processes. We propose that transcriptional dysregulation could be a form of infection infidelity, with certain proteins when expressed inappropriately are capable of triggering pathogen recognition. An interesting aspect of host-pathogen relationship is that subjecting both to stress, as in iron starvation or amino acid limitation elicits responses from both entities to shift the balance of power. In the case of iron starvation, which affects both host and pathogen simultaneously, weakens the latter through induction of metabolic errors and strengthen the former by enabling enhanced recognition of these pathogen mistakes.

Experimental Design: Our previous transcriptomic studies revealed differential expression of the TNF-α signaling pathway in the host when *Chlamydia* was iron-starved using the metal-ion chelator bipyridyl. To explore the hypothesis of infection infidelity, we aimed to induce errors in the bacteria, which we achieved by iron starvation using this chelator. TNF-α levels were measured under iron-starved conditions, and we also examined NFκB nuclear translocation activity to understand the regulation of TNF-α. To investigate differences in pathogen recognition between untreated and error-prone (iron-starved) bacteria, we treated cells with the NOD2 inhibitor GSK717. Given that NOD receptors are activated by bacterial peptidoglycan (PG) components, we concurrently knocked down *amiA*, an amidase involved in peptidoglycan remodeling in *Chlamydia trachomatis* (Ctr), using an inducible CRISPR system. Additionally, we labeled PG to differentiate the remodeling processes among wild-type *Chlamydia*, iron-starved bacteria, and the *amiA* knockdown strain.

Data and results: We made the novel observation that starving *C. trachomatis* of iron, but not tryptophan led to a 10-fold increase in the host pro-inflammatory cytokine TNF-α compared to non-starved controls. This response was found to be NF-κB and NOD2 dependent, as demonstrated by siRNA knockdown of NF-κB and pharmacological inhibition of NOD2 with GSK717. Transcriptomic studies revealed dysregulated induction of expression of the *amiA* gene, which encodes an amidase necessary for peptidoglycan (PG) remodeling during bacterial cell division. *Chlamydia* PG biosynthesis is temporally regulated, with biosynthetic intermediates sequestered and recycled, thus minimizing detection by host PRRs. We hypothesized that dysregulated *amiA* expression leads to enhanced pathogen recognition. Disrupting its iron starvation-induced upregulation via CRISPRi prevented TNF-α expression and NF-κB nuclear translocation. Furthermore, by labeling PG with EDA-DA, which is an analog of D-Ala-D-Ala compatible with Click-It chemistry, we demonstrate a strong correlation between *amiA* overexpression and loss of EDA-DA association with PG, *i.e.* dispersed distribution throughout the host cell cytosol.

Conclusion: These findings indicate that iron starvation induces overexpression of the AmiA amidase to mis localize PG intermediates. Dysregulating production of certain PAMPs by withdrawing iron enhances pathogen recognition. We propose that nutritional immunity induces metabolic errors in the pathogen to enhance recognition by the host, instead of outright killing.

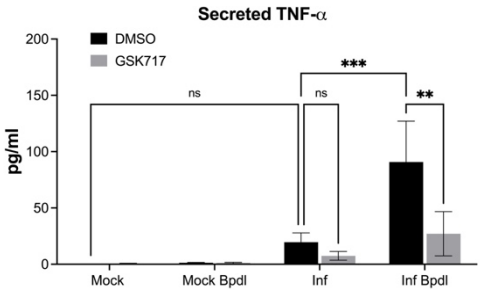

Figure 1. TNF-α levels measured by ELISA in HeLa cells infected with *C. trachomatis* in the presence and absence of Bpdl and GSK717.

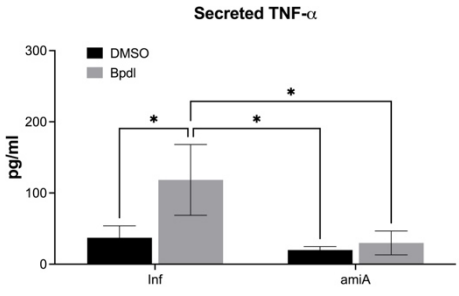

Figure 2. TNF-α levels measured by ELISA in HeLa cells infected with *C. trachomatis* or amiA knockdown strain in the presence and absence of Bpdl.

FROM FIGHTERS TO SUPPRESSORS: NEUTROPHIL REPROGRAMMING INTO GRANULOCYTIC MYELOID-DERIVED SUPPRESSOR CELLS (G-MDSCS) DURING *STAPHYLOCOCCUS AUREUS* BIOFILM INFECTION

Adedayo Ogunware, Zachary Van Roy, Gunjan Kak, & Tammy Kielian

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

Background, Significance, and Hypothesis: Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of immature, anti-inflammatory myeloid cells that accumulate during disease states. MDSCs have been classified into at least two subsets, defined based on cell surface marker expression: granulocytic (G-MDSCs or PMN-MDSCs) and monocytic (M-MDSCs). We recently identified a critical role for G-MDSCs in *S. aureus* biofilm persistence where they comprise 30–60% of the immune infiltrate in the subcutaneous galea in a mouse model of *S. aureus* craniotomy infection, alongside neutrophils (PMNs), which also represent a major population.

Understanding the mechanisms underlying G-MDSC development and function in the context of *S. aureus* biofilm infections is critical to circumvent the anti-inflammatory properties of this population to promote biofilm clearance. Of note, G-MDSCs are not found in the blood during *S. aureus* craniotomy infection but are prevalent in infected tissues, leading to the hypothesis that factors in the tissue milieu are responsible for programming infiltrating peripheral blood PMNs into immune suppressive G-MDSCs. Our prior work has identified mouse G-MDSCs as $CD45^{+}Ly6G^{+}Ly6C^{+}CD11b^{high}$, which is distinct from PMNs ($CD45^{+}Ly6G^{+}Ly6C^{+}CD11b^{low}$). Preliminary evidence from our laboratory suggests that bone marrow-derived PMNs may upregulate CD11b, potentially indicating a transitional state that shifts PMNs toward G-MDSC-like cells during biofilm infections.

Experimental Design and Results: Using multiparametric flow cytometry, *S. aureus* was found to upregulate CD11b expression on PMNs *in vitro*. Interestingly, we identified a novel putative PMN-to-G-MDSC transition state in response to *S. aureus* biofilm, where the $CD11b^{high}$ population also acquired CD14 expression, traditionally a monocytic marker, and exhibited elevated levels of programmed death-ligand 1 (PD-L1) known to inhibit T cell proliferation. These $CD45^{+}Ly6C^{+}Ly6G^{+}CD11b^{high}CD14^{+}$ PMNs significantly suppressed T cell proliferation and displayed impaired *S. aureus* bactericidal activity (Two-way ANOVA), functionally aligning them with G-MDSCs characterized during biofilm infection.

RNA sequencing revealed transcriptional similarities between these $CD45^{+}Ly6C^{+}Ly6G^{+}CD11b^{high}CD14^{+}$ PMNs and *bona fide* G-MDSCs, alongside evidence of a Toll-like receptor 2 (TLR2) signaling signature. We further demonstrated that TLR2 plays a role in the PMN-to-G-MDSC transition, though it is not the sole pathway; PMNs from TLR2 knockout mice showed significantly reduced acquisition of G-MDSC surface markers upon *S. aureus* exposure, whereas MyD88 knockout PMNs, lacking the central adaptor protein for multiple TLRs, failed to acquire G-MDSC characteristics entirely (Two-way ANOVA). This indicates that other receptors upstream of MyD88, besides TLR2, may also be involved and warrant further investigation. Finally, from the bacterial perspective, we found that *S. aureus* proteins ≤30 kDa are primarily responsible for this observed PMN-to-G-MDSC transition.

Conclusion: Contrary to the traditional view of PMNs as terminally differentiated proinflammatory cells dedicated to pathogen clearance, our data reveal that *S. aureus* biofilms can reprogram these cells into immunosuppressive, G-MDSC-like phenotypes. This transition promotes biofilm persistence by suppressing host immune responses. Mechanistically, this reprogramming involves TLR2-MyD88 signaling and specific bacterial-derived factors, although additional pathways remain to be elucidated.

Future studies will focus on identifying the specific *S. aureus* determinants responsible for this PMN-to-G-MDSC transition and other MyD88-dependent pathways involved in PMN reprogramming. These insights may inform the development of host-directed therapies targeting G-MDSC generation to enhance immune clearance of *S. aureus* biofilms.

INTEGRATED STRESS RESPONSE DRIVES IMMUNE DYNAMICS AND BIOFILM PERSISTENCE DURING *S. AUREUS* CRANIOTOMY INFECTION

Artha Govind Lotlikar, Zachary Van Roy, Gunjan Kak, Tammy Kielian

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

Background: Craniotomy is a common neurosurgical procedure involving the removal of a section of the skull (i.e., bone flap) to access the intracranial compartment for the treatment of conditions such as tumors, epilepsy, and cranial bleeds. A serious complication following craniotomy is infection, which occurs in approximately 1-7% of cases. These infections are commonly caused by *Staphylococcus aureus* (*S. aureus*) that forms biofilm on the bone flap surface, which makes the infection recalcitrant to antibiotics and immune clearance. The integrated stress response (ISR) is a cellular stress pathway triggered by factors such as hypoxia, nutrient deprivation, or the unfolded protein response (UPR), with eIF2α phosphorylation (eIF2α-P) acting as the central regulatory hub. The ISR suppresses global protein synthesis while enabling selective translation of genes such as activating transcription factor (ATF4), to promote cell survival and recovery. However, prolonged, or excessive ISR activation may overwhelm its protective capacity, shifting to cell death pathways. Dephosphorylation of eIF2α marks ISR termination and stress resolution.

Significance: Since antibiotics alone do not provide adequate infection clearance, treatment for craniotomy infection often entails multiple additional surgeries, placing additional morbidity on an already medically fragile patient population. This is magnified by the fact that reinfection can occur. To address this, our laboratory has developed a mouse model of *S. aureus* craniotomy infection that mirrors key aspects of human disease, enabling investigation of biofilm persistence mechanisms. Our findings reveal a compartmentalized immune response, with distinct microenvironments shaping context-specific immune regulation. However, the mechanisms underlying the neuropathology of craniotomy infections remain poorly understood and could inform the development of novel immunomodulatory treatment strategies in the face of mounting antimicrobial resistance.

Hypothesis: We hypothesize that the ISR is a key pathway that promotes craniotomy infection persistence and immune dysfunction. Our scRNA-seq data revealed significant induction of ISR-regulated genes during both mouse and human craniotomy infection. Further, eIF2α-P and ATF4 levels are significantly increased in anti-inflammatory granulocytic myeloid-derived suppressor cells (G-MDSCs), neutrophils (PMN), and macrophages exposed to *S. aureus*, all major infiltrates during craniotomy infection. These observations justified further investigation of our hypothesis conducted in this study.

Experimental Design and Results: To confirm ISR activation, Western blots were performed to detect eIF2α-P in the brain and galea (subcutaneous tissue directly above the bone flap) of mice that were subjected to *S. aureus* or sham (no infection) craniotomy. Mice were sacrificed on days 3 and 7, and tissue lysates were analyzed for total and phosphorylated eIF2α. At day 3, eIF2α-P levels were similar between sham and infected mice, but by day 7, eIF2α-P expression was significantly elevated in the infected group. This increase coincided with the timepoint of mature biofilm formation. To assess the role of ISR during *S. aureus* craniotomy infection, mice were treated with an ISR inhibitor (ISRIB; 1 mg/kg/day). Bacterial burdens were significantly higher with ISR inhibition at day 3 compared to vehicle-treated animals (Two-way ANOVA), suggesting that ISR activation aids in controlling early infection. However, by day 7, this protective effect was diminished, suggesting a novel temporal role for ISR activity in dictating infectious outcome. To understand how the ISR influences leukocytes that infiltrate craniotomy infections, macrophages, PMNs, and G-MDSCs were exposed to *S. aureus* planktonic or biofilm cultures. Leukocytes were stained for eIF2α-P and ATF4 after 30 min and 2 h to determine the kinetics of ISR activation. In both *S. aureus* growth conditions, a significant time-dependent increase in eIF2α-P and ATF4 expression of was observed relative to unstimulated cells (Unpaired t-test), confirming ISR induction in response to infection. In sum, these results demonstrate ISR activation in response to *S. aureus*, both *in vivo* and *in vitro*.

Conclusion: Our study identifies the ISR as a key pathway elicited during *S. aureus* craniotomy infection. ISR activation helps control bacterial growth during acute stages, but persistent biofilm growth overwhelms this response by day 7. Ongoing research in our laboratory is focused on understanding how ISR modulation—either inhibition or activation—affects leukocyte phagocytosis and bactericidal activity. We are also investigating the impact of ISR modulation on mitochondrial reactive oxygen species production in response to *S. aureus*, which is an important regulator of leukocyte cell death. This work enhances our understanding of how the ISR influences the immune response and could lead to new therapeutic strategies for managing craniotomy infections.

SERINE PROTEASES MODULATE NEUTROPHIL IMMUNOSUPPRESSION AND SURVIVAL.

Reegan Sturgeon, Sumanas Maram, Ainsley Meyerson, Ridhi Bhola, Esther Johnson, Rakesh K Singh

University of Nebraska Medical Center, Omaha, NE

Background: Tumor-promoting inflammation is a hallmark of cancer, contributing to malignant cells' survival and proliferation. Infiltrating leukocytes and pro-inflammatory cytokines released into the tumor microenvironment (TME) often cause this inflammation. We have previously shown that increased infiltrating neutrophils are associated with pancreatic ductal adenocarcinoma (PDAC) disease progression, which correlates with increased inflammation and neutrophil accumulation.

Significance: Prolonged neutrophil accumulation has been linked to chronic inflammation, which in turn is thought to contribute to tumor progression, therapy resistance, and metastasis.

Hypothesis: In the present study, we examined how neutrophil-PDAC interaction enhances the survival of neutrophils dependent on serine proteases and their inhibitors. Previous reports suggest that serine proteases, such as cathepsin G, can contribute to inflammation and induce caspase-independent neutrophil apoptosis.

Experimental Design: We examined the role of serine proteases and their inhibitors on neutrophil survival using defined cellular models.

Results: We observed an association between increased serine protease and neutrophil survival. Moreover, this survival was modulated by cellular aggressiveness and therapy resistance. In addition, we observed increased survival of neutrophils associated with inhibition of serine proteases and increased expression of serpins to proteases in neutrophils upon PDAC cancer cell conditioned media treatment.

Conclusion: In summary, these studies point to neutrophil serine proteases and their inhibitors as versatile mediators that modulate neutrophil survival, leading to an immunosuppressive microenvironment and identifying them as potential targets for therapeutic interventions.

TRIPLE NEGATIVE BREAST CANCER CELLS ACQUIRE LYMPHOCYTE PROTEINS AND GENOMIC DNA DURING TROGOCYTOSIS WITH T CELLS

Anutr Sivakoses, Haley Q. Marcarian, Anika M. Arias, Allison R. Lam, Juan A. Santamaria, Geoffrey C. Gurtner, Alfred L.M. Bothwell

Trogocytosis is the process by which a recipient cell siphons small membrane fragments and proteins from a donor cell and may be utilized by cancer cells to avoid immune detection. We observed lymphocyte specific protein expressed by TNBC cells via immunofluorescence imaging of patient samples. Image analysis of CD45RA expression, a T cell specific protein, revealed that all stages of TNBCs express CD45RA. Flow cytometry revealed TNBC cells trogocytose CD45 protein from T cells. We also showed that the acquisition of these lymphoid markers is contact dependent. Confocal and super-resolution imaging further revealed $CD45^{+}$spherical structures containing T cell genomic DNA inside TNBC cells after co-culture. Trogocytosis between T cells and TNBC cells altered cancer cell gene expression. Our results revealed that CD45 is obtained by TNBC cells from T cells via trogocytosis and that TNBC cells express CD45 intracellularly and on the membrane.

RENAL CANCER CELLS ACQUIRE IMMUNE SURFACE PROTEIN THROUGH TROGOCYTOSIS AND HORIZONTAL GENE TRANSFER

Haley Q. Marcarian, Anutr Sivakoses, Anika M. Arias, Olivia C. Ihedioha, Benjamin R. Lee, Maria C. Bishop, Alfred L.M. Bothwell

Trogocytosis is an underappreciated phenomenon that shapes the immune microenvironment surrounding many types of solid tumors. The consequences of membrane-bound proteins being deposited from a donor immune cell to a recipient cancer cell via trogocytosis are still unclear. Here, we report that human clear cell renal carcinoma tumors stably express the lymphoid markers CD45, CD56, CD14, and CD16. Flow cytometry performed on fresh kidney tumors revealed consistent CD45 expression on tumor cells, as well as varying levels of the other markers mentioned previously. These results were consistent with our immunofluorescent analysis, which also revealed colocalization of lymphoid markers with carbonic anhydrase 9 (CAIX), a standard kidney tumor marker. RNA analysis showed a significant upregulation of genes typically associated with immune cells in tumor cells following trogocytosis. Finally, we show evidence of chromosomal DNA being transferred from immune cells to tumor cells during trogocytosis. This horizontal gene transfer has transcriptional consequences in the recipient tumor cell, resulting in a fusion phenotype that expressed both immune and cancer specific proteins. This work demonstrates a novel mechanism by which tumor cell protein expression is altered through the acquisition of surface membrane fragments and genomic DNA from infiltrating lymphocytes. These results alter the way in which we understand tumor-immune cell interactions and may reveal new insights into the mechanisms by which tumors develop. Additionally, further studies into trogocytosis will help push the field towards the next generation of immunotherapies and biomarkers for treating renal cell carcinoma and other types of cancers.