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25th Annual Upstate New York Immunology Conference

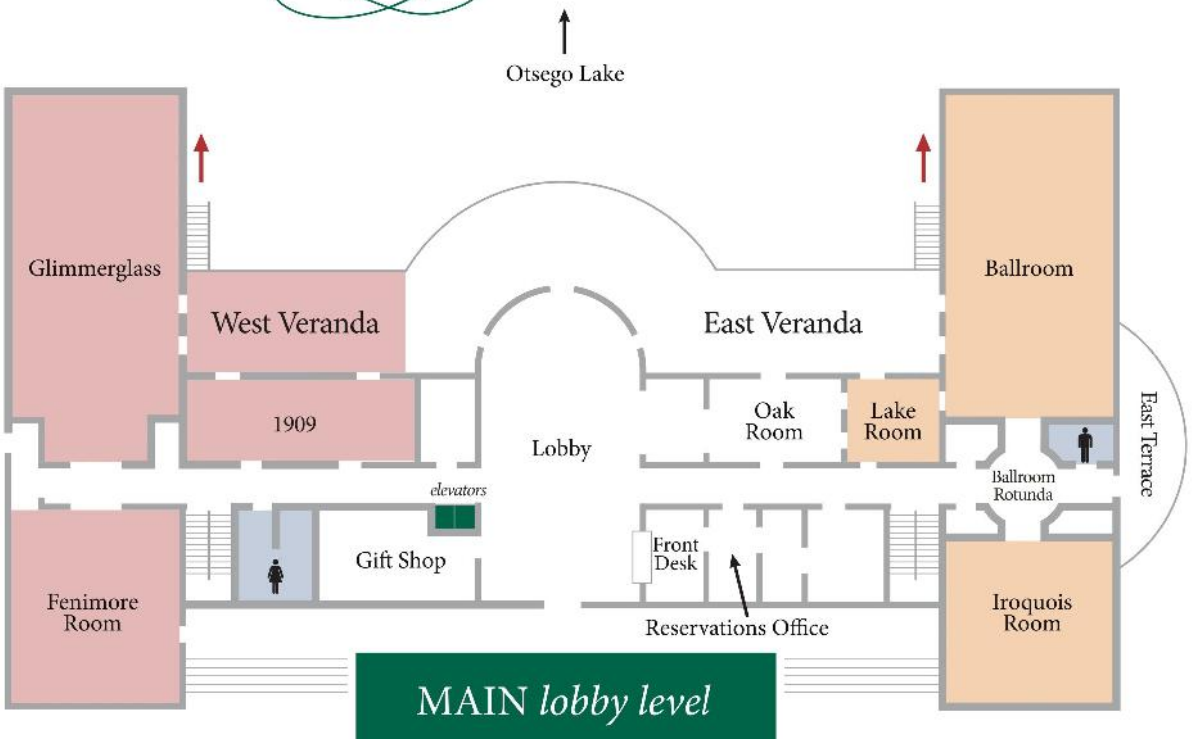
American Association of Immunologists

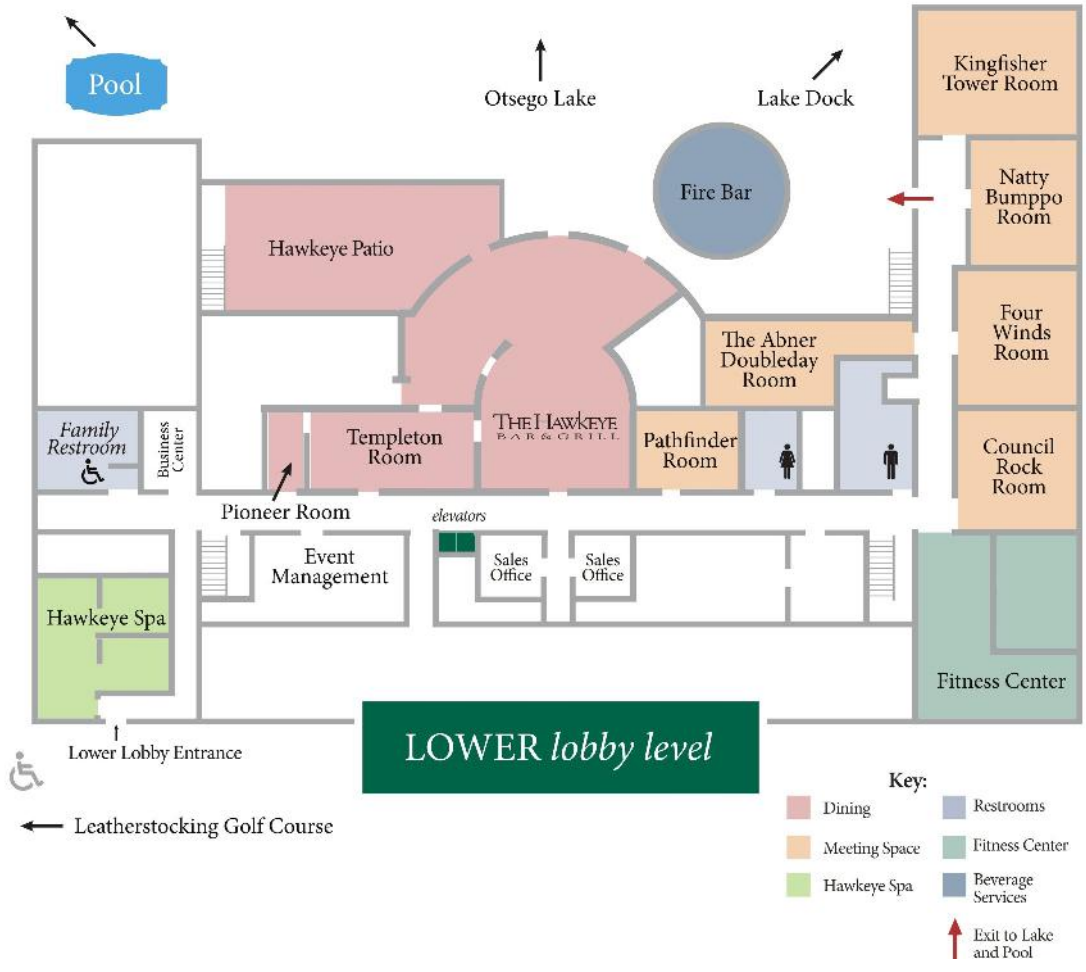
Young investigator Awards

The Otesaga Hotel and Resort
60 Lake St.
Cooperstown, NY

October 16-19, 2023

the Otesaga Event Spaces





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NYIC 2022
Table of Contents

Congratulations to NYIC Underrepresented Minority Travel Award Winners!

Isabel Marie Forlastro
Cornell University

Erin Evonne Jean
University of Pennsylvania

Viviana Maymi
Cornell University

UPSTATE NEW YORK IMMUNOLOGY CONFERENCE (NYIC)

The Upstate New York Immunology Conference (NYIC) started in 1997 as a small retreat to facilitate interactions among young scientists, institutions, and renowned experts in the field of Immunology. In just a few short years, the number of attendees grew and a larger venue was needed to meet the future needs of the Conference. The Otesaga Resort Hotel's more central location in Cooperstown, NY, has provided an opportunity for others to attend. There will be free time in the schedule to explore Cooperstown on your own. Main Street is just a short stroll from the hotel. Weather permitting, you can take advantage of the Otesaga fire bar and engage fellow researchers in informal discussions. Trainees will also have an opportunity to win an iPad during one of two drawings. You must be present at the drawing to win!

The American Association of Immunologists (AAI) is once again providing ten (10) Young Investigator Awards with the amount supplemented by NYIC.
NYIC is also providing ten (10) Trainee Travel Awards. All award winners also give short Oral Presentations based on their poster abstracts.

Our Keynote speaker **Dr. Eugene Oltz** (The Ohio State University). In addition, Dr. Oltz will be giving a workshop.

It's celebration time! NYIC is the perfect event to engage trainees and seasoned scientists...and so much more. Collaborations happen here, and so does celebrating achievements in science. This year is no exception. Join in on the **25th Anniversary Gala** where everyone is invited to put on their dancing shoes and spend time with colleagues at the photo both.

While all these elements lend to the overall atmosphere, one simple principle goal of this Conference remains. To provide an opportunity for young and senior scientists to gather in a setting that is diverse enough to meet the needs of all attendees yet remain small enough to allow for personal interactions. While always challenging, it is the goal of the NYIC Scientific Advisory Board and NYIC Conference Organizers to give graduate students and postdoctoral fellows the opportunity to present their research and engage in conversations that will stimulate further discussions, collaborations, and interest in pursuing a new or different way of looking at their research.

THE OTESAGA

Since its opening in 1909, ***The Otesaga Resort Hotel*** has been a destination people couldn't wait to see and experience. The Clark family, who owns the hotel to this day, commissioned architect Percy Griffin to design The Otesaga. The 400 windows that wrap around the Otesaga provide stunning views of Lake Otsego. An architectural feature common today that was considered exciting when built is the width of the driveway. It was uncommon in those days for driveways to be wide enough for two cars (or carriages) to easily pass one another. The hotel has continued to maintain its original aura of charm and gracious hospitality, while also growing and changing with the times. The Otesaga is now an AAA Four-Diamond hotel and belongs to the Historic Hotels of America.

We hope you share our enthusiasm and enjoy your time with us!

Upstate New York Immunology Conference
Schedule of Events
October 16-19, 2022

It is up to each attendee's personal preference whether to mask while in public areas.

Monday, October 16th

Time	Event	Location
2:00-4:00 p.m.	Conference Registration	Iroquois
3:00-5:00 p.m.	Hotel Check-in	Lobby
5:00-6:00 p.m.	Welcome Reception	Oak Room
6:00-8:00 p.m.	Plated Dinner	Glimmerglass
8:15 p.m.	Welcome to NYIC 2023	
8:20 p.m.	Introduction: Richard Robinson, PhD	

Keynote Presentation
Sponsored by ***BioLegend, Inc.***

Eugene Oltz, PhD
Chair, Department of Microbial Infection and Immunity
Program Administrator, Master of Science in Immunology
and Microbial Pathogenesis, and PhD in Immunology and Immunotherapeutics
The Ohio State University

"Wiring Schemes for Innate and Adaptive Lymphoid Cells"

9:30 p.m.	Leisure gatherings	Hawkeye Bar

Tuesday, October 17th

Time	Event	Location
7:00-8:15 a.m.	**Breakfast Buffet at Leisure**	Glimmerglass
8:20-8:30 a.m.	**Morning Announcements**	Ballroom
8:30-10:00 a.m.	***Symposium I: Insights to Immune System Regulation*** Chair: Eugene Oltz, PhD	Ballroom
8:30-9:00	**David Langlais, PhD** (McGill University) *"Multiomics Probing of Defective Immunity"*	
9:00-9:30	**Liya Ding, PhD** (Roswell Park) *"Immune-mediated Therapeutic Resistance to PARP Inhibition in Ovarian Cancer"*	
9:30-10:00	**Tim Mosmann, PhD** (University of Rochester) *"Mining High-dimensional Flow Cytometry Data for Clinical Predictions"*	
10:00-10:15 a.m.	**Beverage Break** (available 9-11am)	Iroquois
10:15-11:15 a.m.	***Symposium II: New Concepts in B Cell Funtion*** Chair: Joel Wilmore, PhD	Ballroom
10:15-10:45	**Jim Drake, PhD** (Albany Medical College) *"MCH Class II Conformer—Opposite Sides of the Same Coin"*	
10:45-11:15	**Chang-Wang Jerry Lio, PhD** (Ohio State) *"Epigenetic Remodeling by Vitamin C Potentiates Plasma Cell Differentiation"*	
11:15-12:30 a.m.	**Workshop - Dr. Oltz** *"Writing Tips for Scientific Manuscripts and Specific Aims Pages"*	Ballroom
12:30-1:30 p.m.	Lunch Buffet	Glimmerglass

Tuesday, October 17th

Time	Event	Location
2:00-3:30 p.m.	***Symposium III: Tissue-specific Immunity*** Chair: Deborah Fowell, PhD	Ballroom
2:00-2:30	**Roberto M. Alers-Velazquez, PhD** (Dartmouth College) *"Neuroimmune Response to Subclinical Neonatal Herpes Simplex Virus-1 Infection"*	
2:30-3:00	**Nathan Roy, PhD** (SUNY Upstate) *"Integrin Signaling Events that Control T-cell Migration"*	
3:00-3:30	**Elizabeth Wohlfert, PhD** (University at Buffalo) *"Muscling Through Chronic Infection"*	
3:30-4:00 p.m.	Corporate Presentation Introduction: Jesse Bonin BD Biosciences **Dan McDonald -** Single Cell Multiomics Sales Specialist **"***TBA"*	
4:00-6:30 p.m.	Free Time	
6:30-7:30 p.m.	**Dinner Buffet**	Glimmerglass
7:30-8:00 p.m.	Corporate Presentation Introduction: Jesse Bonin BioLegend, Inc. **Nathan Lucas -** Technical Application Scientist	
8:00-11:00 p.m.	**Leisure time with colleagues**	Hawkeye Bar

Wednesday, October 18th

Time	Event	Location
7:00-8:00 a.m.	Breakfast Buffet at Leisure	Glimmerglass
8:00-8:25 a.m.	All Oral Poster Presentations to be Uploaded	Kingfisher/Council Rock
8:25-8:30 a.m.	Morning Announcements	Kingfisher/Council Rock
8:30-10:00 a.m.	**Oral Poster Presentations**	Kingfisher

Group A - ***Adaptive Immunity in Infection and Disease***

Chairs: Rich Robinson and Joel Wilmore

8:30-8:45 **Katya McDonald, B.A.** (University of Rochester) Ø
"Identification of T cell exhaustion as a biomarker of Staphylococcus aureus persistent osteomyelitis in humanized mice" (P4, page 62)

8:45-9:00 **Achamaporn Punnanitinont, Ph.D.** (University at Buffalo) Ø
"TLR7 activation of age-associated B cells mediates disease in a mouse model of primary Sjogren's disease" (P9, page 67)

9:00-9:15 **Zachary T. Hilt, Ph.D.** (Cornell University) Ø
"Retinoic Acid is Required for the Sequestration of CD8+ T Cells in the Brain During Congenital Cytomegalovirus Infection" (P15, page 73)

9:15-9:30 **Marlena R. Merling, B.S.** (Ohio State) Ø
"The emergence of SARS-CoV-2 lineages and associated saliva antibody responses among asymptomatic individuals in a large university community" (P33, page 91)

9:30-9:45 **Victoria Zoccoli-Rodriguez,** (SUNY Upstate) ♦
"Gut IgA+ Plasma Cells Differentially Utilize Metabolism to Regulate Antibody Secretion" (P47, page 105)

Ø AAI/NYIC Young Investigator Award ♦ NYIC Trainee Travel Award

Wednesday, October 18th

Time	Event	Location
8:30-10:00 a.m.	**Oral Poster Presentations** **Group B—New Insights in Immune Regulation** Chairs: Eyal Amiel and Kate MacNamara	Council Rock
8:30-8:45	**Rachel Grazda, B.A.** (Albany Medical College) Ø *"Resolvin E1 improves efferocytosis and rescues severe aplastic anemia in mice" (P5, page 63)*	
8:45-9:00	**Brian G. Morreale, M.S.** (Roswell Park) Ø *"Myeloid Cell Reprogramming through De Novo Pyrimidine Synthesis Blockade " (P11, page 69)*	
9:00-9:15	**Bay Vagher, B.S.** (University of Vermont) ♦ *"UDP-glucose Regulates Dendritic Cell Mitochondrial Respiration via a Nitric Oxide-dependent Mechanism"* *(P13, page 71)*	
9:15-9:30	**Ikechukwu B. Jacob, M.S.** (SUNY Upstate) ♦ *"Differential Immunoregulatory Roles of Surfactant Protein A Variants in SARS-CoV-2 Infected Humanized Transgenic Mice" (P19, page 77)*	
9:30-9:45	**Thejaswini Giridharan, M.S.** (University at BUffalo) ♦ *"Complement signaling as a T-cell checkpoint in the ovarian cancer tumor microenvironment " (P50, page 108)*	

Ø AAI/NYIC Young Investigator Award ♦ NYIC Trainee Travel Award

9:45-10:15 a.m.	Beverage Break	Kingfisher Foyer
10:15-11:45 a.m.	**Oral Poster Presentations** **Group C – Cancer Immunotherapy** Chairs: Scott Gerber and Jason Muhitch	Kingfisher
10:15-10:30	**Maggie L. Lesch, B.S.** (University of Rochester) Ø *"Stimulating the Type I Interferon Pathway to Enhance the Efficacy of Short Course Radiotherapy in Rectal Cancer " (P18, page 76)*	

Wednesday, October 18th

Time	Event	Location

10:30-10:45 **John P. Miller, M.S.** (University of Rochester) ♦
"Investigating the role of HE4-mediated immunosuppression in ovarian cancer " (P22, page 80)

10:45-11:00 **Nicholas J. Salgia, B.S.** (Roswell Park) ♦
"Sarcomatoid Dedifferentiation Promotes Immunogenic Properties in Renal Cell Carcinoma" (P36, page 94)

11:00-11:15 **Gary Hannon, Ph.D.** (University of Rochester) Ø
"Treating Metastatic Pancreatic Ductal Adenocarcinoma with SBRT and IL-12 mRNA" (P45, page 103)

11:15-11:30 **Shannon L. Ferry, B.S.** (Dartmouth College) ♦
"Car T cells armored with Super IL2 Nd IL33 reshpe the tumor microenvironment to control solid tumor growth" (P55, page 113)

Ø AAI/NYIC Young Investigator Award ♦ NYIC Trainee Travel Award

10:15-11:45 p.m. **Oral Poster Presentations** Council Rock
Group D – Regulating Innate Immunity
Chairs: Beth Wohlfert and Efthymia Iliana Matthaiou

10:15-10:30 **E. Evonne Jean, B.S.** (University of Pennsylvania) Ø
"Cutaneous Immunity Against Helminth Infection Requires IL-33 Expressing APCs" (P3, page 61)

10:30-10:45 **Amber Bahr, B.S.** (Albany Medical College) Ø
"Type I IFNs and eosinophils in Ehrlichia-induced liver Injury" (P7, page 65)

10:45-11:00 **Tao Yu, Ph.D.** (Cornell University) ♦
"NLRP3 S-palmitoylation by ZDHHC7 Promotes Inflammasome Activation in Macrophages" (P14, page 72)

11:00-11:15 **Ronzon Shihab, B.A.** (University at Buffalo) ♦
"Amphiregulin Treatment Promotes Satellite Cell and Fibro-adipogenic Progenitor Functions during Chronic T. gondii Infection" (P35, page 93)

Wednesday, October 18th

Time	Event	Location
11:15-11:30	**Audrey Roy-Dorval, B.S.** (McGill University) ♦ "*Analysis of lipid uptake and lipid droplets in primary murine Group 2 Innate Lymphoid Cells" (P43, page 101)*	

Ø AAI/NYIC Young Investigator Award ♦ NYIC Trainee Travel Award

11:45-12:00 p.m.	**Display Odd Number Posters**	Ballroom
12:00-1:30 p.m.	Lunch Buffet and Awards Presentation	Glimmerglass
1:30-5:00 p.m.	Cash bar	Ballroom Rotundra
1:30-3:00 p.m.	**Poster Session Odd No. Posters/Vendor Fair**	Ballroom
3:00-3:15 p.m.	**Remove Odd No. Posters/Display Even No.**	Ballroom
3:15-4:45 p.m.	**Poster Session-Even No. Posters/Vendor Fair**	Ballroom
4:45-5:00 p.m.	**Remove Posters and iPad Drawing**	Ballroom
5:00-7:00 p.m.	Free time for attendees	

Let the Celebration Begin!!

7:00-9:00 p.m.	**Hot Entrée and Charcuterie Boards** (Cash Bar - Ballroom Rotundra)	Iroquois Room
7:00-10:00 p.m.	LaValley DJ and Big Shot Photo Booth	Ballroom
9:30-10:30 p.m.	Dessert and Coffee	Iroquois Room

Thursday, October 19th

Time	Event	Location
7:00-9:30 a.m.	**Breakfast Buffet at Leisure Hotel Check-out**	Glimmerglass
9:50 a.m.	**Morning Announcements**	Ballroom
10:00-11:30 a.m.	***Symposium IV: Clinical Immunology*** Chair: Mike Robek	Ballroom

10:00-10:30 **Andrew Flyak, Ph.D.** (Cornell University)
"Structures of Human Antibodies Bound to Multiple Epitopes on HCV Glycoprotein E2 Inform Vaccine Design Efforts"

10:30-11:00 **William Lee, Ph.D.** (Wadsworth/NYSDOH)
"Immunology in a Public Health Setting"

11:00-11:30 **Rebekah Honce, Ph.D.** (University of Vermont)
"Therapeutic Antibody Development for RNA Viruses of Pandemic Potential"

11:45-12:00 p.m.	**Closing Remarks & iPad Drawing**	Ballroom
12:00-1:00 p.m.	**Lunch Buffet**	Glimmerglass

Depart from Conference

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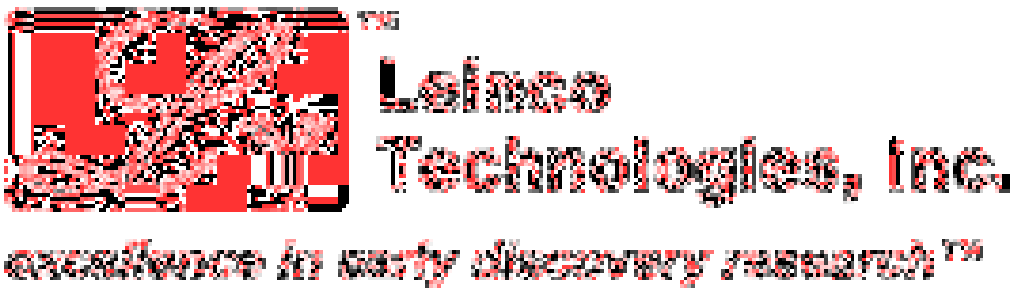

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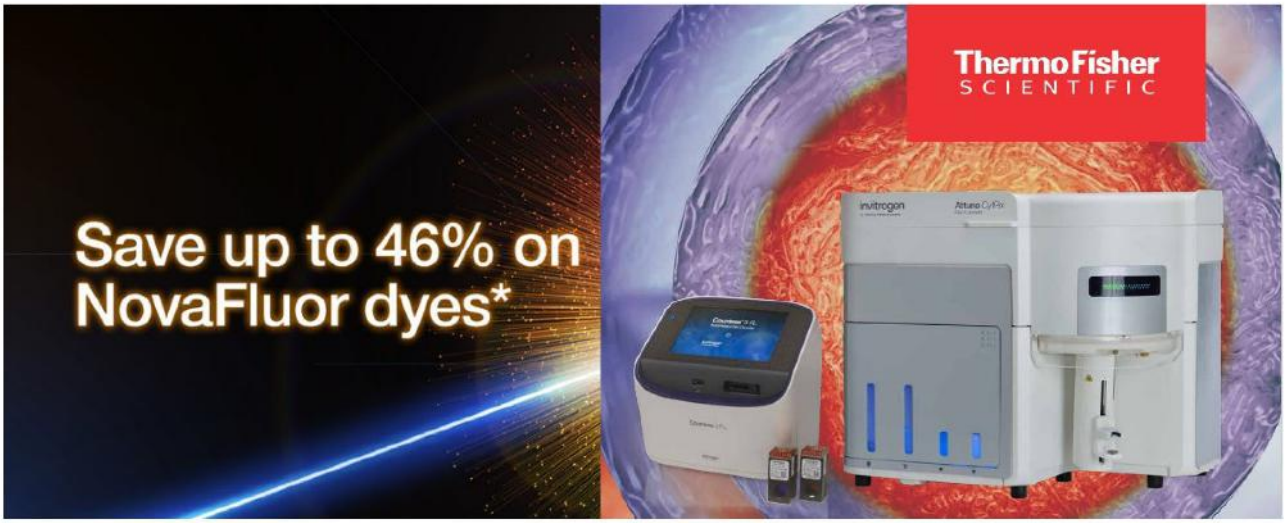

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In recognition of the significance
of this meeting and work being done by
Graduate Students
and Postdoctoral Fellows,
The American Association
of Immunologists
has provided
Ten (10) Young Investigator Awards.

Each will also have the opportunity
to present their research as a poster
and give a brief talk.

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2022 NYIC

Keynote Speaker

Eugene Oltz Ph.D.

Chair, Department of Microbial Infection and Immunity

Program Administrator, Master of Science in Immunology and Microbial Pathogenesis, and PhD in Immunology and Immunotherapeutics

Ohio State University

"Wiring Schemes for Innate and Adaptive Lymphoid Cells"

Current projects in the Oltz Lab aim to determine the roles of transcription, chromatin states and architectural proteins in orchestrating developmental programs of the lymphoid cells that compose our innate and adaptive immune systems. Our goal is to identify the key factors and pathways that endow each cell type with its signature functions, then leveraging the information to engineer better cellular therapeutics for cancer and chronic inflammatory diseases. In addition, we apply our approaches to understand epigenetic mechanisms that sculpt the diverse repertoire antigen receptors required for B and T lymphocytes to recognize a veritable universe of pathogens and, the same time, allow these cells to respond rapidly to DNA damage during the assembly of B and T cell receptors. Finally, our laboratory has initiated studies to profile immune responses mounted against SARS-CoV-2, as part of our Serological Sciences Center of Excellence, called STOP-COVID, and the national SeroNet community.

Symposium I

Insights to Immune System Regulation

Chair: Dr. Eugene Oltz

"Multiomics Probing of Defective Immunity"

David Langlais, PhD

Inflammation Genomics Lab, Dept of Human Genetics,
Dahdaleh Institute of Genomics Medicine, McGill University

The differentiation and activation status of immune cells is tightly controlled by the combinatorial effect of an array of transcriptional regulators. Failure from the transcription factors to establish and maintain an appropriate epigenomic landscape, and thus transcriptional program, may lead to immunodeficiencies and susceptibility to infectious diseases. Our laboratory uses different models and functional genomics approaches to investigate the role of various transcription factors in maintaining a healthy and efficient immune system. This presentation will focus on our identification of IRF1 as a new gene causing Mendelian susceptibility to mycobacterial disease and our dissection of its function at the cellular and genomic level.

"Immune-mediated Therapeutic Resistance to PARP Inhibition in Ovarian Cancer"

Liya Ding, PhD

Roswell Park Comprehensive Cancer Center

The tumor suppressor gene *BRCA1* (Breast cancer susceptibility gene 1) maintains genomic stability and suppresses tumorigenesis by promoting DNA double-strand break (DSB) repair through homologous recombination (HR). Mutations or defects in the *BRCA1* gene is frequently found in ovarian, breast, pancreatic and prostate cancers. Based on the concept of synthetic lethality between poly (ADP-ribose) polymerase (PARP) inhibition and BRCA deficiency, PARP inhibitors (PARPi) have been developed for the treatment of BRCA-deficient tumors. PARP inhibition has dramatically changed the treatment landscape of advanced ovarian tumors with BRCA mutations, however, acquired resistance to PARPi remains a major challenge in the clinic. It is critical to understand the resistance mechanisms and develop new strategies to enhance the clinical use of PARP inhibition in ovarian cancer. By using genetically engineered mouse models (GEMMs) of Brca1-deficient ovarian cancer, we uncovered an adaptive resistance mechanism to PARP inhibition mediated by the tumor associated macrophages (TAMs) in the tumor microenvironment (TME). Mechanistically, PARP inhibition induced the STAT3 signaling pathway in tumor cells, which in turn promoted pro-tumor polarization of tumor associated macrophages (TAMs) in the TME of ovarian cancer. Ablation of STAT3 in tumor cells mitigated polarization of M2-like pro-tumor macrophages in the TME and increased tumor-infiltrating T cells in response to PARP inhibitor treatment. These findings are corroborated in patient-derived, PARPi-resistant BRCA1-mutant ovarian tumors. We further demonstrated that STING agonists reshape the immunosuppressive TME by reprograming suppressive myeloid cells into an antitumor status in the TME of PARPi-resistant ovarian tumors and overcome the TME-dependent adaptive resistance to PARPi in ovarian cancer. This effect is further enhanced by addition of PD-1 blockade. Taken together, our study elucidates the importance of targeting innate immune suppression to improve the therapeutic efficacy of PARPi in BRCA1-mutant ovarian tumors.

"Mining High-dimensional Flow Cytometry Data for Clinical Predictions"

Tim Mosmann
University of Rochester, Rochester, NY

Abstract Not Provided

Symposium II

New Concepts in B Cell Function

Chair: Dr. Joel Wilmore

"MHC Class II Conformers – Opposite Sides of the Same Coin"

Jim Drake, PhD

Department of Immunology and Microbial Disease, Albany Medical College

The function of MHC class II molecules is multi-dimensional. They present antigen-derived peptides to CD4 T cells to engage the T cell receptor and CD4 to drive T cell activation. Class II molecules are also signaling molecules and can drive activation of antigen presenting cells such as B cells. Initial studies on mAb-driven MHC class II signaling revealed a difference in the signaling properties of two anti-I-A^k mAbs. Follow-up studies revealed that the two antibodies recognize distinct conformers of the I-A^k class II molecule, formed by the differential pairing of transmembrane domain GxxxG dimerization motifs (i.e., M1- vs. M2-paired class II) and exhibiting differential partitioning into membrane lipid domains. Subsequent studies revealed differential peptide loading of the two class II conformers, with peptide derived from BCR-bound antigen being selectively loaded onto lipid raft-associated M1-paired class II molecules. Our most recent work extends this paradigm to human class II by identifying and characterizing a mAb that selectively binds lipid raft-resident M1-paired HLA-DR molecules. Unpublished work suggests that either mAb or T cell receptor engagement of M1- vs. M2-paired class II drives different levels of B cell activation. These findings significantly expand our understanding of MHC class II immunobiology and suggest ways that MHC class II signaling impacts overall immune responsiveness.

"Epigenetic Remodeling by Vitamin C Potentiates Plasma Cell Differentiation"

Heng-Yi Chen[1#], Ana Almonte-Loya[1,2#], Fang-Yun Lay[1], Michael Hsu[1], Eric Johnson[2], Edahi Gonzalez-Avalos[2], Jieyun Yin[2], Richard S. Bruno[3], Qin Ma[4,6], Hazem E. Ghoneim[1,6], Daniel J. Wozniak[1], Fiona E. Harrison[5], Chan-Wang Jerry Lio[1,2,6*]

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Ascorbate (vitamin C) is an essential micronutrient in humans. The chronic severe deficiency of ascorbate, termed scurvy, has long been associated with increased susceptibility to infections. How ascorbate affects the immune system at the cellular and molecular levels remained unclear. From a micronutrient analysis, we identified ascorbate as a potent enhancer for antibody response by facilitating the IL-21/STAT3-dependent plasma cell differentiation in mouse and human B cells. The effect of ascorbate is unique, as other antioxidants failed to promote plasma cell differentiation. Ascorbate is especially critical during early B cell activation by poising the cells to plasma cell lineage without affecting the proximal IL-21/STAT3 signaling and the overall transcriptome. As a cofactor for epigenetic enzymes, ascorbate facilitates TET2/3-mediated DNA modification and demethylation of multiple elements at the *Prdm1* locus. DNA demethylation augments STAT3 association at the *Prdm1* promoter and a downstream enhancer, thus ensuring efficient gene expression and plasma cell differentiation. The results suggest that an adequate level of ascorbate is required for antibody response and highlight how micronutrients may regulate the activity of epigenetic enzymes to regulate gene expression. Our findings imply that epigenetic enzymes can function as sensors to gauge the availability of metabolites and influence cell fate decisions.

This research was funded by LJI/KKR independent investigator fund; NIH National Cancer Institute K22 (K22CA241290); startup funds from the Department of Microbial Infection and Immunity and from the Pelotonia Institute of Immuno-oncology at the Ohio State University (all to C.-W.J.L.).

Workshop

"Writing Tips for Scientific Manuscripts and Specific Aims Pages"

Eugene Oltz, Ph.D.

Symposium III

Tissue-Specific Immunity

Chair: Dr. Deborah Fowell

"Neuroimmune Response to Subclinical Neonatal Herpes Simplex Virus-1 Infection"

Roberto M. Alers-Velazquez, Ph.D.
Geisel School of Medicine at Dartmouth

Neonatal herpes simplex virus (nHSV) infections cause infant mortality and lifelong behavioral and neurological pathologies in survivors. These include psychomotor retardation, seizure disorders, cognitive and learning disorders, and possibly neurodegenerative diseases. We have developed a holistic approach using a novel mouse model to study nHSV that encompasses infection, cytokine changes in the CNS, and behavioral studies. Understanding the nHSV infection's influence on a healthy, developing brain is crucial to understanding the psychobiological impact of neuroinvasive diseases. We have shown that a subclinical nHSV infection causes anxiety-like behavior in neonatally infected mice, which can be significantly decreased when the dam is immunized against HSV-1. Due to the importance of the neuroimmune response against viral infection, we investigated cytokines/chemokines involved in the induction of a pro-inflammatory state of microglia and astrocytes. Our data reveal a subset of cytokines/chemokines associated with pro-inflammatory microglia/astrocyte response involved in neuroinflammation and neurodegeneration. The upregulation of microglial pro-inflammatory markers further supported this data. This project seeks to characterize the mechanism of HSV-1 neuroimmune modulation and neurological sequelae on neonates by investigating the neuroinflammatory response to nHSV, defining the role of microglia and astrocytes against nHSV, and characterizing the correlation between nHSV infection and behavior morbidity from a neuroimmune point of view.

"Integrin signaling events that control T cell migration"

Nathan Roy
SUNY Upstate Medical University

Leukocyte migration out of the vasculature into peripheral tissue is crucial for their role in fighting pathogens, promoting tissue repair, and attacking solid tumors. This process is a key control point in the inflammatory response and relies on integrin interactions with their endothelial ligands. Importantly, integrin ligation triggers signaling events within leukocytes that drive cytoskeletal changes, cell polarization, and ultimately efficient migration. However, the molecular mechanisms of how integrin ligation leads to the appropriate cytoskeletal responses are largely unknown. Here, we have identified two adaptor proteins, CrkL and CasL, that are critical factors controlling T cell migration downstream of integrin engagement. Primary mouse T cells lacking either protein show reduced actin polymerization in response to ICAM-1, and have severe migratory defects revealed by live-cell imaging. Interestingly, the molecular mechanism behind the similar migratory phenotype of the two knockouts is markedly different. While CrkL controls PI3K activity in the front of migrating cells, CasL seems to be involved in maintaining proper cortex/membrane integrity via RhoA signaling. These data highlight the role of adaptor proteins in facilitating integrin signaling events, and reveal new pathways that could be manipulated to alter T cell function.

"Muscling through Chronic Infection"

Monica Humby, Ronzon Shihab, Caleb Buerger, Kirk Personius and Elizabeth A. Wohlfert

Department of Microbiology and Immunology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY 14203, Department of Rehabilitation Science, School of Public Health and Health Professions, University at Buffalo, Buffalo, NY 14203

After a tissue is infected, the invading pathogen must be controlled and tissue repaired or else long-term morbidity and mortality will ensue. *Toxoplasma gondii* infections result in a non-resolving chronic infection with tissue cysts residing preferentially in skeletal muscle and the central nervous system. In contrast to the brain, little is known about the immune response and immune-regulation in the muscle in *Toxoplasma.* Damaged muscles normally repair themselves in part by formation of restorative macrophages (MΦ) and activation of myogenic progenitor cells. Our work revealed that restorative MΦ do not develop in *Toxoplasma*-infected muscles and that SC are unable to be activated. How this happens is unknown but critical to the understanding of muscle repair mechanisms during chronic infections and the long-term implications of chronic infection. Many cells, both immune and stromal are important to the overall outcome of tissue repair in skeletal muscle. We have found that Tregs surprisinginly acquire a tissue-injurious function and impair the ability of muscle to repair itself. We have developed multiple tools to decipher how this cellular network functions and what is altered. This talk will focus on our recent findings in the balance of chronic infection and muscle homeostasis.

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Technical Application Scientist / Team Lead

BioLegend, Inc.

"Stimulation with a Superagonistic Anti-CD28 Antibody Shows Treg Expansion and Provides an In Vitro Model for Immunotherapeutic Research"

Summary: CD28 is a cell surface protein expressed on T cells that provides a costimulatory signal to enhance T cell activation and proliferation when bound in the presence of a cognate ligand from an antigen presenting cell. Using a number of methodologies related to BioLegend reagents, we have developed and characterized a CD28 superagonistic clone that leads to the preferable expansion of Tregs and we hope will play a critical role in the advancement of T cell activation and differentiation as well as a useful tool in manipulating this pathway to control immune responses in various disease states.

Oral Poster Presentations

8:30—10:00 a.m.
Kingfisher

Group A

Adaptive Immunity in Infection and Disease

Chairs: Rich Robinson & Joel Wilmore

8:30-8:45 **Katya McDonald, B.A.** (University of Rochester) Ø
"Identification of T cell exhaustion as a biomarker of Staphylococcus aureus persistent osteomyelitis in humanized mice" (P4, page 62)

8:45-9:00 **Achamaporn Punnanitinont, Ph.D.** (University at Buffalo) Ø
"TLR7 activation of age-associated B cells mediates disease in a mouse model of primary Sjogren's disease" (P9, page 67)

9:00-9:15 **Zachary T. Hilt, Ph.D.** (Cornell University) Ø
"Retinoic Acid is Required for the Sequestration of CD8+ T Cells in the Brain During Congenital Cytomegalovirus Infection" (P15, page 73)

9:15-9:30 **Marlena R. Merling, B.S.** (Ohio State) Ø
"The emergence of SARS-CoV-2 lineages and associated saliva antibody responses among asymptomatic individuals in a large university community" (P33, page 91)

9:30-9:45 **Victoria Zoccoli-Rodriguez,** (SUNY Upstate) ♦
"Gut IgA+ Plasma Cells Differentially Utilize Metabolism to Regulate Antibody Secretion" (P47, page 105)

Ø AAI/NYIC Young Investigator Award
♦ NYIC Trainee Travel Award

Oral Poster Presentations

8:30-10:30 a.m.
Council Rock

Group B
New Insights in Immune Regulation

Chairs: Eyal Amiel & Kate MacNamara

8:30-8:45 **Rachel Grazda, B.A.** (Albany Medical College) Ø
"Resolvin E1 improves efferocytosis and rescues severe aplastic anemia in mice" (P5, page 63)

8:45-9:00 **Brian G. Morreale, M.S.** (Roswell Park) Ø
"Myeloid Cell Reprogramming through De Novo Pyrimidine Synthesis Blockade " (P11, page 69)

9:00-9:15 **Bay Vagher, B.S.** (University of Vermont) ♦
"UDP-glucose Regulates Dendritic Cell Mitochondrial Respiration via a Nitric Oxide-dependent Mechanism"
(P13, page 71)

9:15-9:30 **Ikechukwu B. Jacob, M.S.** (SUNY Upstate) ♦
"Differential Immunoregulatory Roles of Surfactant Protein A Variants in SARS-CoV-2 Infected Humanized Transgenic Mice" (P19, page 77)

9:30-9:45 **Thejaswini Giridharan, M.S.** (University at BUffalo) ♦
"Complement signaling as a T-cell checkpoint in the ovarian cancer tumor microenvironment " (P50, page 108)

Ø AAI/NYIC Young Investigator Award
♦ NYIC Trainee Travel Award

Oral Poster Presentations

1:30-3:00 p.m.
Kingfisher

Group C

Cancer Immunotherapy

Chair: Scott Gerber

10:15-10:30 **Maggie L. Lesch, B.S.** (University of Rochester) Ø
"Stimulating the Type I Interferon Pathway to Enhance the Efficacy of Short Course Radiotherapy in Rectal Cancer "
(P18, page 76)

10:30-10:45 **John P. Miller, M.S.** (University of Rochester) ♦
"Investigating the role of HE4-mediated immunosuppression in ovarian cancer " (P22, page 80)

10:45-11:00 **Nicholas J. Salgia, B.S.** (Roswell Park) ♦
"Sarcomatoid Dedifferentiation Promotes Immunogenic Properties in Renal Cell Carcinoma" (P36, page 94)

11:00-11:15 **Gary Hannon, Ph.D.** (University of Rochester) Ø
"Treating Metastatic Pancreatic Ductal Adenocarcinoma with SBRT and IL-12 mRNA" (P45, page 103)

11:15-11:30 **Shannon L. Ferry, B.S.** (Dartmouth College) ♦
"Car T cells armored with Super IL2 Nd IL33 reshape the tumor microenvironment to control solid tumor growth"
(P55, page 113)

Ø AAI/NYIC Young Investigator Award
♦ NYIC Trainee Travel Award

Oral Poster Presentations

1:30-3:00 p.m.
Kingfisher

Group D

Regulating Innate Immunity

Chairs: Beth Wohlfert &
Efthymia Iliana Matthaiou

10:15-10:30 **E. Evonne Jean, B.S.** (University of Pennsylvania) Ø
"Cutaneous Immunity Against Helminth Infection Requires IL-33 Expressing APCs" (P3, page 61)

10:30-10:45 **Amber Bahr, B.S.** (Albany Medical College) Ø
"Type I IFNs and eosinophils in Ehrlichia-induced liver Injury" (P7, page 65)

10:45-11:00 **Tao Yu, Ph.D.** (Cornell University) ♦
"NLRP3 S-palmitoylation by ZDHHC7 Promotes Inflammasome Activation in Macrophages" (P14, page 72)

11:00-11:15 **Ronzon Shihab, B.A.** (University at Buffalo) ♦
"Amphiregulin Treatment Promotes Satellite Cell and Fibro-adipogenic Progenitor Functions during Chronic T. gondii Infection" (P35, page 93)

11:15-11:30 **Audrey Roy-Dorval, B.S.** (McGill University) ♦
" *Analysis of lipid uptake and lipid droplets in primary murine Group 2 Innate Lymphoid Cells" (P43, page 101)*

Ø AAI/NYIC Young Investigator Award
♦ NYIC Trainee Travel Award

2023 NYIC Poster Listing

Ø AAI/NYIC Young Investigator Award ♦ NYIC Trainee Travel Award

Poster 1.

Preliminary Binding Studies Between the ITK_SH2 domain and SLP76 Based Peptides

Authors: Shaik, Ruqiyah[1]; Bah, Alaji[1]
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The use of allogenic hematopoietic stem cell transplantation (allo-HSCT) has shown to be an effective method of treatment for hematological malignancies. However, the presence of mature donor T-cells, while mediating graft-versus tumor (GVT) responses, also leads to the occurrence of graft-versus-host-disease (GVHD). Inhibition of the T-cell protein, interleukin-2-inducible T-cell kinase (ITK), and its interaction with its adaptor protein, SH2 domain-containing leukocyte protein of 76 kDa (SLP76), has been shown to uncouple GVT from GVHD. Currently, small molecule kinase inhibitors are being used as an alternative treatment to chemotherapy, yet the use of these inhibitors pose challenges due to lack of specificity. To increase specificity of inhibitors, the intricacies of interaction between ITK and SLP76 must first be understood. As of yet we know that the SH2 domain of ITK binds to a disordered region on SLP76 which contains a tyrosine at position 145 (Y145). We hypothesize that the ITK_SH2 domain binds tightly to SLP76, with dissociation constant (Kd) in nM range, and that this interaction is mediated by phosphorylation of Y145. Using binding studies, the mechanism and specificity of the interaction between ITK and SLP76 can be further elucidated. To investigate this interaction, we will utilize binding techniques such as Isothermal Titration Calorimetry and fluorescence-based binding to study the strength and mechanism of this interaction. Our preliminary results indicate binding between the ITK_SH2 domain and a SLP76 based peptide in vitro. We will then screen the ITK_SH2-SLP76 complex using novel inhibitors to test for disruption of binding. The results of our studies could set the stage for the development of novel and specific ITK-SLP76 inhibitors.

Poster No. 2

The bovine mammosphere-derived epithelial cell secretome modulates innate immune cell functions

Authors: Rebecca M. Harman1, Anja S. Sipka2, Kelly A. Oxford1, Gerlinde R. Van de Walle1
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2College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA

Mastitis in dairy cattle is a common and costly disease. It is primarily caused by bacterial infection and treated using conventional antibiotics (abx). Because the overuse of abx leads to the evolution of abx-resistant bacterial strains, and abx don't contribute to the repair of mastitis-damaged udder tissue, there is a need for alternative therapies for this condition.

Bovine mammosphere-derived epithelial cell (MDEC) cultures are enriched for mammary stem and progenitor cells. We have previously shown that the secretome of bovine MDECs, consisting of all bioactive factors released by the cells, can be collected and delivered as conditioned medium (CM). In vitro, bovine MDEC CM (i) inhibits the growth of mastitis-causing bacteria and (ii) acts on endothelial and epithelial cells in ways that could support tissue repair, prompting us to propose MDEC CM as an alternative therapy for mastitis in cattle.

In this in vitro study, we evaluated the immunomodulatory effects of bovine MDEC CM on bovine neutrophils, innate immune cells critical for resolving mastitis. We demonstrated that MDEC CM stimulates neutrophil chemotaxis and phagocytosis, while decreasing intracellular ROS and extracellular trap formation, when compared to neutrophils exposed to control medium. Using siRNAs, we determined that the secretome factors C-X-C motif chemokine 6, peroxiredoxin-2 and catalase contribute to the immunomodulatory effects of the bovine MDEC secretome on neutrophils.

These data enhance our knowledge of the bioactive factors in the bovine MDEC secretome, which will help determine if it may be an effective alternative therapy for mastitis in dairy cattle.

Poster 3.

Cutaneous Immunity Against Helminth Infection Requires IL-33 Expressing APCs

Authors: E. Evonne Jean[1], De'Broski Herbert[2]
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[2]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Interleukin 33 (IL-33) is an alarmin cytokine thought to be solely released from damaged epithelia that is essential for protection against gastrointestinal (GI) helminth (worm) infection, but whether IL-33 derived from hematopoietic cell lineages serves any role in cutaneous immunity remains unclear. Our recent work demonstrates that CD11c+ myeloid antigen presenting cells can produce IL-33 in a biologically important manner that shapes Type 2 immunity. Herein, we developed a percutaneous infection model using the GI nematode Strongylodies ratti to better understand the role for IL-33 in cutaneous immunity against larval stages. Data show that C57BL/6 mice develop resistance to percutaneous infection upon secondary challenge in an IL-33 dependent manner. Surprisingly, mice with a selective IL-33 deficiency only in myeloid antigen presenting cells (APCs) (CD11cCre) also failed to develop secondary resistance to S. ratti, suggesting myeloid-derived IL-33 is essential for cutaneous immunity. Mechanistically, we find that loss of myeloid IL-33 impairs recruitment of both gd (gamma delta) T cells and CD4+ T cells that express the IL-33 receptor ST2. Furthermore, gene deficient mice that lack all gdT cells or selective antibody depletion of CD4+ T cells show impaired immunity against S. ratti indicating an essential role for these lymphocyte populations in IL-33 dependent acquired resistance. Unexpectedly, mice lacking the Type 2 transcription factor STAT6 (Signal transducer and activator of transcription 6), surprisingly have no defects in primary or secondary cutaneous immunity, indicating that IL-33 drives a non-canonical Type 2 host protective response. Our work implies that myeloid APCs are a necessary source of IL-33 that drives acquired immunity against helminths, potentially through regulating gdT cell and CD4+ T cell effector function(s). Ongoing studies seek to understand how myeloid APC derived IL-33 controls recruitment and activation of gdT cells and CD4+ T cells to promote cutaneous immunity.

Poster 4.

Identification of T cell exhaustion as a biomarker of *Staphylococcus aureus* persistent osteomyelitis in humanized mice

Katya McDonald[1,2], Motoo Saito[2], Javier Rangel-Moreno[2], John Owen[3], Edward Schwarz[1,2], Stephen Kates[3], and Gowrishankar Muthukrishnan[1,2]

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[2]Center for Musculoskeletal Research and Department of Orthopedics, University of Rochester Medical Center, Rochester, NY, USA

[3]Department of Orthopaedic Surgery, Virginia Commonwealth University, Richmond, VA, USA

Staphylococcus aureus, a significant human pathogen, continues to be the leading cause of implant-associated osteomyelitis including peri-prosthetic joint infections and fracture-related infections. It is broadly considered incurable due to recalcitrant biofilms and colonization of the osteocyte-lacuno canalicular network (OLCN) of cortical bone, which cannot be eradicated with standards of care short of amputation. However, it is also known that patients can resolve acute infections and live a full life with asymptomatic *S. aureus* osteomyelitis. Unfortunately, currently available diagnostics to guide conservative vs. aggressive surgical treatment options for patients are very limited. This led the 2018 International Consensus Meeting on Musculoskeletal Infection to conclude that developing a functional definition for acute vs. chronic osteomyelitis is the greatest priority in this field.

To this end, preclinical natural history studies evaluated transitions in host immunity and found that initial robust pro-inflammatory responses in the acute phase of infection transition from Th1 and Th17 to suppressive Treg adaptive immune responses over time. To account for human-specific *S. aureus* pathology, we developed a humanized mouse model of osteomyelitis and showed that the commencement of persistent osteomyelitis (14 days post-infection) occurs with large numbers of proliferating $CD3^{+}$/$Tbet^{+}$ adjacent to purulent abscesses in the bone marrow. This coincided with increased infection and osteolysis, suggesting that human T cell infiltration and proliferation in the bone do not aid bacterial clearance. Subsequent multi-omics studies in an improved humanized NSG-SGM3 BLT model revealed that: 1) human T cells are remarkably heterogenous in gene expression and numbers, and 2) immune checkpoint proteins are upregulated in Th1 and Th17 cells due to infection suggesting an exhaustion phenotype. Moreover, in a clinical pilot study, these proteins were upregulated in the serum of patients with chronic *S. aureus* osteomyelitis. Remarkably, increased levels of these immune checkpoint proteins were highly predictive of adverse outcomes such as arthrodesis, reinfection, amputation, and septic death in these patients. Our results indicate that T cell exhaustion could be a functional biomarker for persistent *S. aureus* osteomyelitis and treatment outcome.

Poster No. 5

Resolvin E1 improves efferocytosis and rescues severe aplastic anemia in mice

Authors: Rachel A. Grazda[1], Allison N. Seyfried[1,2], and Katherine C. MacNamara[1]
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Current treatments for severe aplastic anemia (SAA) rely on hematopoietic stem cell (HSC) transplantation and immunosuppressive therapies, however these treatments are not always effective. While immune-mediated destruction and inflammation are known drivers of SAA, the underlying mechanisms that lead to persistent inflammation are unknown. Using an established mouse model of SAA, we observed a significant increase in apoptotic cells within the bone marrow (BM) and demonstrate impaired efferocytosis in SAA mice, as compared to radiation controls. Single-cell transcriptomic analysis revealed heterogeneity among BM monocytes and unique populations emerged during SAA characterized by increased inflammatory signatures and significantly increased expression of *Sirpa* and *Cd47*. CD47, a "don't eat me" signal, was increased on both live and apoptotic BM cells, concurrent with markedly increased expression of signal regulatory protein alpha (SIRPa; alpha) on monocytes. Functionally, SIRPa (alpha) blockade improved cell clearance and reduced accumulation of CD47-positive apoptotic cells. Lipidomic analysis revealed a reduction in the precursors of specialized pro-resolving lipid mediators (SPMs) and increased prostaglandins in the BM during SAA, indicative of impaired inflammation resolution. Specifically, 18-HEPE, a precursor of E-series resolvins, was significantly reduced in SAA-induced mice relative to radiation controls. Treatment of SAA mice with Resolvin E1 (RvE1) improved efferocytic function, BM cellularity, platelet output, and survival. Our data suggest that impaired efferocytosis and inflammation resolution contributes to SAA progression and demonstrate that SPMs, such as RvE1, offer new and/or complementary treatments for SAA that do not rely on immune suppression.

Poster No. 6

Beta-Hydroxybutyrate Attenuates Allergen-Induced Responses of Human Bronchial Smooth Muscle

V. Amanda Fastiggi*[1,2], Madeleine M. Mank[1], Paola E. Peña-García[1,2], Jennifer L. Ather[1], Sophia H. Piffard[3], Kalev Freeman[3] and Matthew E. Poynter[1]

[1]Department of Medicine, Division of Pulmonary Disease and Critical Care, and The Vermont Lung Center, [2]Cellular, Molecular, and Biomedical Sciences doctoral program, [3]Department of Emergency Medicine. University of Vermont, Burlington, VT, 05405, USA

Asthma is a heterogenous lung syndrome characterized by hyperresponsiveness to bronchoconstrictive triggers, airway inflammation, and lung remodeling. House Dust Mite (HDM) allergens are the most common cause of allergic asthma and initiate innate and adaptive immune responses by triggering cells through multiple pathways, including protease-activated receptor 2 (PAR2). HDM also induces pro-inflammatory cytokine production from bronchial smooth muscle that can amplify and prolong asthmatic responses. We recently reported that HDM induces the contraction of human bronchial smooth muscle cells (HBSMC), causing the surface area in vitro to become markedly condensed. Recent work has provided evidence of the therapeutic potential of ketogenic diets and ketone supplementation in several pathological conditions, including neurological diseases, diabetes, cancer, and asthma. Ketogenesis results in the production of the ketone body, beta-hydroxybutyrate (BHB), which has anti-inflammatory properties, including immunosuppressive activity on the NLRP3 inflammasome, an important innate immune mechanism driving asthma exacerbation, as well as antioxidant and anti-excitotoxic properties. We have reported that in vivo dietary approaches to augment BHB concentrations are associated with and sufficient to mitigate the hyperresponsive bronchoconstriction manifest in multiple preclinical asthma models. Herein, we used HBSMCs in vitro to explore mechanisms underlying the bronchoprotective effects of BHB. Quantitation of cellular surface area and ELISAs revealed that BHB was sufficient to significantly diminish the HDM-induced pathological contraction of HBSMC and the production of pro-inflammatory cytokines irrespective of the BHB enantiomer. Single (R- or S-) BHB enantiomers or a racemic (R,S-) BHB mixture equally inhibited HDM protease activity in a dose-dependent manner. To determine whether BHB attenuated PAR2-mediated contraction, HBSMC were exposed to a synthetic PAR2 agonist in the absence or presence of increasing concentrations of BHB. Our results indicate that BHB dose-dependently attenuated PAR2-mediated HBSMC contraction. As dietary BHB augmentation is sufficient to mitigate the bronchoconstrictive trigger-induced hyperresponsiveness of preclinical models of inherent asthma in which allergens are not present, we examined whether BHB could mitigate HBSMC contraction induced by other bronchoconstriction-inducing agonists. Quantitation of cellular surface area from images acquired using video microscopy, as well as using an HBSMC-embedded collagen lattice assay, demonstrated that HBSMC contraction was significantly inhibited by BHB in a dose-dependent manner. As these agonists do not elicit effects through PAR2, we speculate that BHB may provoke apical effects such as calcium flux, downstream of receptor interactions, to inhibit the signaling cascade inducing HBSMC contraction. To determine if BHB could inhibit HBSMC contraction through attenuation of calcium flux, intracellular live video microscopy of cells loaded with a calcium-sensitive dye was performed. Results from these studies demonstrate that BHB significantly inhibited agonist-induced calcium flux. These results indicate that increasing systemic BHB concentrations through dietary interventions could provide symptom relief for several asthma endotypes through apical effects on bronchial smooth muscle signaling, including PAR2 activation and calcium flux inhibition, thereby decreasing agonist-induced pro-inflammatory cytokine production, contraction, and bronchoconstriction.

Poster No. 7

Type I IFNs and eosinophils in *Ehrlichia*-induced liver injury

Authors: Amber Bahr and Katherine C. MacNamara, PhD
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The underlying mechanisms that drive pathology in infection-induced shock are not well understood. The tick-borne pathogen *Ixodes ovatus erhlichia* (IOE) induces severe inflammation that drives vascular damage, liver injury, and death. We identified a critical role for type I interferons (IFNs) in IOE-induced shock. Mice that lack the receptor for type I interferons, or receptors for both type I and II IFNs, improves survival by 50-70 and 90%, respectively. IOE infection drives a significant increase in circulating granulocytes, including neutrophils, eosinophils, and basophils. Within the bone marrow (BM), however, eosinophils and basophils were increased in IOE-infected wildtype mice whereas neutrophils were reduced suggesting production is shifted to these rarer granulocyte populations during acute phase of IOE infection. Furthermore, expansion of rare BM granulocytes was abrogated in *Ifnar*-deficient mice implicating IFNAR signaling in production of these rare granulocyte populations. Analysis of granulocyte infiltrates in the liver during IOE infection demonstrated eosinophils were highly active and undergoing degranulation as determined by increased CD69 (marker for activated eosinophils) and CD63 (marker for degranulation) expression. Eosinophil activation was dependent on type I IFNs as the phenotype was not observed in the livers from *Ifnar*- and *Ifnar; Ifngr*-deficient mice. Moreover, WT eosinophils display increased 7-aminoactinomycin D (7AAD) binding during infection suggesting eosinophils are selectively undergoing cell death in the liver. The direct effect of type I IFNs on recruitment of granulocytes to the liver was evaluated in radiation-induced BM chimeric mice (WT: *Ifnar-/-* chimeras), where we observed that IFNAR signaling provided a recruitment advantage into the liver for eosinophils, whereas IFNAR signaling was dispensable for recruitment of other granulocyte subsets. To distinguish the location of recruited cells we performed intravenous labeling and determined that eosinophils were located within the liver parenchyma. Together, these data suggest type I IFNs are modulating hepatic eosinophils to become proinflammatory through increased degranulation and cell death thus contributing to robust hepatic inflammation and exacerbating liver injury.

Poster No. 8

Mast cell interaction with nanoparticles in the context of skin allergy

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In recent years, there has been an increase in the number of engineered nanomaterials (ENMs) in the workplace and consumer products, causing concern in environmental, occupational, and consumer health and safety. Due to their small size (1 to 100nm) and unique properties, they can be used in multiple applications ranging from electronics to drug delivery, thereby increasing their exposure. They can enter the body and alter the innate immune reaction by interacting with effector immune cells, either exacerbating or suppressing the response. Previous studies using the mouse contact hypersensitive model (CHS) showed that 20nm silica nanoparticles (Si 20nm) suppressed the allergic reaction when applied within 2h post-challenge. In contrast, ~50 nm manganese-doped titanium dioxide nanoparticles ($mTiO_2$) exacerbated the response. These interactions with the immune system suggest a connection with mast cells. Mast cells are tissue-resident immune cells that are crucial to allergic responses. They can sense a multitude of environmental exposures and danger signals during the early stages of allergen stimulation leading to the recruitment and activation of other immune cells. However, the specific mechanisms ENMs use to influence mast cell activation are poorly understood.

To address this knowledge gap, an ongoing study is being conducted to evaluate the combinatorial effects of the potent 2,4-dinitrofluorobenzene (DNFB) hapten sensitizer with Si 20nm and $mTiO_2$ nanoparticles using bone marrow-derived mast cells (BMMC). The effects of these compounds on cytotoxicity, cytokine secretion, and degranulation are being assessed. Preliminary results show that Si 20nm does not influence mast cell activation or viability. In contrast, $mTiO_2$ and DNFB increase mast cell degranulation while decreasing viability. These findings suggest that Si 20nm may suppress the immune response by blocking mast cell degranulation, thereby reducing the amount of proinflammatory cytokines released. Further work will include studying the levels of cytokine release and investigating the interaction of skin cells with mast cells. This approach aims to determine the effects of nanoparticles on their exchange within the immune system and the skin microenvironment.

Poster No. 9

TLR7 activation of age-associated B cells mediates disease in a mouse model of primary Sjogren's disease

Achamaporn Punnanitinont [1], Eileen M. Kasperek [1], Chengsong Zhu [2], Guan Yu [3, *], Jeffrey C. Miecznikowski [3] and Jill M. Kramer [1]

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Primary Sjogren's disease (pSD, also referred to as Sjogren's syndrome) is an autoimmune disease that primarily occurs in women. In addition to exocrine gland dysfunction, pSD patients exhibit B cell hyperactivity. B cell-intrinsic TLR7 activation is integral to the pathogenesis of SLE, a disease that shares similarities with pSD. The role of TLR7-mediated B cell activation in pSD, however, remains poorly understood. We hypothesized that age-associated B cells (ABCs) were expanded in pSD mice and that TLR7 -stimulated ABC subsets exhibited pathogenic features characteristic of disease. Our data revealed that ABC expansion and TLR7 expression were enhanced in a pSD mouse model in a Myd88-dependent manner. Splenocytes from pSD mice showed enhanced sensitivity to TLR7 agonism as compared to those derived from controls, and pSD splenocytes secreted heightened levels of IgG, IgG2c, and IFN gamma following TLR7 ligation. Sort-purified marginal zone (MZ) B cells and ABCs from pSD mice showed enhanced inflammatory cytokine secretion and were enriched for anti-nuclear autoantibodies following TLR7 agonism. Finally, IgG from pSD patient sera showed elevated reactivity for numerous autoantigens that were secreted preferentially by TLR7-stimulated murine MZ B cells and ABCs. Thus, these data indicate pSD B cells are hyper-responsive to TLR7 agonism and TLR7-activated B cells contribute to pSD through cytokine and autoantibody production. Therapeutics that target TLR7 signaling cascades in B cells may have utility in pSD patients.

Poster No. 10

Investigating the alterations of gut macrophages in iron overload mice and their impact on ferrophilic *Yersinia* infection

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Hemochromatosis is a genetic or nongenetic disorder characterized by excess iron accumulation in the body. Hereditary hemochromatosis (HH) occurs from mutations in genes that encode components required for hepcidin synthesis and is characterized by hepcidin reduction and increased iron recycling from macrophages. Secondary hemochromatosis, often acquired from medical treatment such as frequent blood transfusion, results in increases in hepcidin levels and increases in intracellular iron. This phenomenon can drive irregularities in immune cells, leading to dysregulation of the immune system and increased susceptibility to ferrophilic bacterial infections. However, the distinct molecular mechanism for this occurrence remains unknown. The aim of this work is to study the impact of iron overload on intestinal macrophages, specifically studying the transition of macrophages from a proinflammatory, antimicrobial phenotype into an anti-inflammatory, tissue-remodeling phenotype after ferrophilic *Yersinia* infection. Iron overload is achieved by keeping C57Bl/6 mice on a high iron diet for three weeks, and a clinical *Yersinia enterocolitica* isolate (Ye ATCC 27729), is used as the model strain for ferrophilic infection. Three weeks on the high iron diet causes significantly higher iron levels in the serum and liver of the iron overloaded mice (termed FeB6) compared to WT B6 mice. Iron overload is also characterized by infiltration of myeloid cells into the intestine and colon, including a significantly higher population of macrophages in the FeB6 intestines. Within the small intestine and colon the monocyte-macrophage waterfall is impaired during iron overload, resulting in significantly higher numbers of P1 monocytes ($Ly6C^+$ $CCR2^+$ $F4/80^-$) and P2 transitioning monocytes/immature macrophages ($Ly6C^+$ $F4/80^+$) in FeB6 mice. These cell populations have a more inflammatory phenotype than mature P3/P4 macrophages ($Ly6C^-$ $CCR2^-$ $F4/80^+$). It is considered that imbalance in these populations can drive complications within the gut. The predicted outcome of this accumulation of infiltrating/transitioning monocytes is increased proinflammatory cytokine release and reduced tissue remodeling. The alterations in macrophage population and phenotype have a detrimental impact on iron overload mice during infection with Ye, illustrated by the improved body weight, survival, and cell profiles of macrophage depleted mice.

Poster No. 11

Myeloid Cell Reprogramming through *De Novo* Pyrimidine Synthesis Blockade

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Immune checkpoint inhibitors (ICIs), such as those that engage PD-1 on cytotoxic $CD8^+$ T cells, can improve survival outcomes across multiple solid cancer types. However, in several cancers, such as triple-negative breast cancer (TNBC), the use of these agents either achieve modest benefits similar to standard-of-care or are effective in only some subsets of patients. Therefore, while ICIs are promising, there are many barriers which limit ICI efficacy, including the immune suppressive tumor microenvironment (TME). One prominent immune suppressive cell population of the TME are myeloid-derived suppressor cells (MDSC), which are generated in the bone marrow by tumor-derived signals. MDSCs are immature myeloid cells that inhibit the proliferation or effector functions of $CD8^+$ T cells even with the use of ICI agents. To overcome this obstacle, our laboratory developed a novel approach to target MDSC biogenesis in the bone marrow to mitigate production and function to bolster ICI activity in preclinical models of TNBC. We identified a metabolic susceptibility in MDSCs and targeted that vulnerability using agents known as dihydroorotate dehydrogenase (DHODH) inhibitors. DHODH inhibitors block *de novo* pyrimidine metabolism and are being used as an anti-AML therapy to promote the maturation of leukemic myeloid progenitors. We found that combining DHODH and PD-1 blockade significantly diminished TNBC growth and metastasis, with reduced MDSC suppressive activity. We hypothesized that DHODH blockade reprograms MDSCs and renders them less pro-tumorigenic. To test this hypothesis, we made use of both loss- and gain-of-function approaches. First, in mice receiving the combination regimen, MDSC depletion using an antibody-based approach lessened the therapeutic benefit, suggesting that DHODH blockade acts through and functionally reprograms MDSCs. Secondly, using an 'add-back' approach, MDSCs generated in response to DHODH blockade reduced tumor growth compared to MDSCs derived from vehicle controls. Altogether, these data indicate that DHODH blockade dampens the pro-tumorigenic phenotype of MDSCs, suggesting a novel approach to reprogram MDSCs for use in combination immunotherapies.

Poster No. 12

SIRT2 inhibition protects chronic inflammatory disease by regulating gut epithelial barrier integrity

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Chronic inflammatory diseases, such as Inflammatory Bowel Disease (IBD), pose a significant global health threat. However, the intricate mechanisms underpinning the progression of IBD remain insufficiently explored. Previous research utilizing genetic methods have shown that the depletion of a vital NAD+-dependent protein lysine deacylase, sirtuin 2 (SIRT2), promoted the process of IBD, whereas other study reported pharmacological inhibition of SIRT2 could inhibit IBD severity, which showed conflicting roles of SIRT2 in IBD progression. Here, we validated these inconsistent results by using two distinct SIRT2 inhibitors (TM and AGK2) and a PROTAC SIRT2 degrader. Our investigation firmly confirmed SIRT2 as a promising target for IBD treatment. Importantly, we demonstrated that the protective effects of SIRT2 inhibitors were not attributed to off-target effects, as both TM and AGK2 exhibited similar protective effects in a murine model of IBD induced by Dextran Sodium Sulfate (DSS). Mechanistically, the inhibition of SIRT2 by these compounds suppressed ARF6-mediated E-cadherin endocytosis and recycling, thus protecting the intestinal epithelial integrity and gut epithelium barrier, which contributed to the protective effect in IBD. Furthermore, the results from our investigation indicated that the PROTAC SIRT2 degrader, similar to the genetic knockout of SIRT2, didn't show a protective role in the IBD development. We believe the differential effects of small molecule inhibitors and genetic knockout are due to the fact that the SIRT2 inhibitors only inhibit some but not all the activities of SIRT2, which further emphasized the importance of substrate-dependent inhibitors development in IBD treatment. Overall, our study provided an interesting example that genetic knockout and pharmacological inhibition were not consistent, and this mechanistic understanding further support SIRT2 as a promising target for treating IBD.

Poster No. 13

UDP-glucose Regulates Dendritic Cell Mitochondrial Respiration via a Nitric Oxide-dependent Mechanism

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Dendritic cells (DCs) are key innate immune cells that fine tune and orchestrate immune responses beginning when they first encounter microbial molecules via cell surface receptors such as Toll-Like Receptors (TLRs). TLR stimulation induces the activation process denoted by rapid and widespread changes in the DCs metabolic profile accompanied by large scale changes in gene expression. One of these notable genes is *Nos2*, which encodes inducible nitric oxide synthase (iNOS), an enzyme that produces gaseous molecule nitric oxide (NO). NO first acts as a potent antimicrobial agent, however, at high levels it also exerts cytotoxicity through inhibition of vital cellular activities including mitochondrial respiration. Our lab has shown there is a discrete level of TLR stimulus and consequently NO, that fully inhibits mitochondrial respiration. Therefore, we set out to investigate which metabolites or signaling pathways can modulate this threshold, then study the ramifications of this modulation on cellular metabolism and survival. In this work, we characterized the role of glycogen precursor, UDP-glucose, as an autocrine signaling molecule through the $P2Y_{14}$ receptor in DCs by assessing their proinflammatory phenotypes, iNOS protein abundance, NO production, mitochondrial respiration via real time extracellular flux analysis, and the subsequent consequence on cellular survival. We show that the UDP-glucose/$P2Y_{14}$R axis regulates iNOS at both the genetic and functional level, and thus results in modulation of the mitochondrial respiration threshold favoring earlier inhibition, and exacerbated cell death in low stimulus environments. These studies show that DCs use the UDP-glucose/$P2Y_{14}$R/iNOS axis to bolster proinflammatory phenotypes in low stimulus environments helping us to better model physiological responses to microbes.

Poster No. 14

NLRP3 *S*-palmitoylation by ZDHHC7 Promotes Inflammasome Activation in Macrophages

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Abstract: Multiple pathogen and damage-associated molecular patterns can induce NACHT-, leucine-rich-repeat- (LRR), and pyrin domain-containing protein 3 (NLRP3)-mediated inflammasome activation in macrophages. Hyperactivation of NLRP3 inflammasome contributes to a variety of human chronic inflammatory diseases. Understanding how NLRP3 inflammasome is regulated can potentially provide new strategies to treat inflammatory diseases. Here, we demonstrated that NLRP3 is *S*-palmitoylated on linker-domain by palmitoyl-transferase ZDHHC7 in macrophages. Perturbation of NLRP3 linker-domain *S*-palmitoylation by modification site mutation, ZDHHC7 depletion, or pharmacratic ZDHHC7 inhibition dramatically decreases ASC oligomerization, Caspase-1 and Gasdermin D (GSDMD) cleavage, and IL-1beta secretion in mouse primary macrophages. Similarly, ZDHHC7 disruption also inhibits GSDMD cleavage and IL-1beta secretion in human macrophages. Furthermore, ZDHHC7 knockout or inhibition significantly inhibited IL-1beta and IL-18 secretion in an endotoxic shock mouse model, suggesting NLRP3 *S*-palmitoylation is also vital for inflammasome activation *in vivo*. Mechanistically, ZDHHC7-mediated NLRP3 linker-domain *S*-palmitoylation promotes NLRP3 localization at the *trans*-Golgi network under resting state and facilitates NLRP3 interaction with downstream adaptors upon activation, ultimately promoting the formation of NLRP3 inflammasome complex. Therefore, our study identifies a new regulatory mechanism of NLRP3 activation and may provide a potential therapeutic strategy to treat NLRP3-related human disorders.

Poster No. 15

Retinoic Acid is Required for the Sequestration of CD8+ T Cells in the Brain During Congenital Cytomegalovirus Infection

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Congenital cytomegalovirus (CMV) infection occurs in up to 2.2% of births, making it the most common congenital infection in the United States. Brain abnormalities are a frequent clinical outcome in congenital CMV infections that can lead to vision impairment, hearing loss, and motor/cognitive defects. We previously reported that CD8+ T cells use the CCR9/CCL25 axis to migrate into the brain after infection to control viral replication. However, the molecules responsible for the upregulation of CCR9 on CD8+ T cells infiltrating the brain are unknown. Previous work has identified retinoic acid as a key factor that promotes expression of CCR9 on CD8+ T cells. However, all of this work has been in the context of CD8+ T cells homing to the gut.

To determine whether retinoic acid is required for CD8+ T cells migration to the brain, we performed adoptive transfer experiments with donor cells that express a dominant negative retinoic acid receptor. Interestingly, we found CD8+ T cells require retinoic acid signaling to efficiently enter the brain during congenital CMV and failure to see retinoic acid results in the inability of CD8+ T cells to form tissue residency. We also sought to determine the cells that are responsible for producing retinoic acid during CMV infection. For these studies, we focused our attention on immune cells in the cervical lymph node (CLN), since this is the site of priming for CD8+ T cell migrating to the brain. We observed a significant increase in the number of dendritic cells that produced retinoic acid compared to uninfected animals. We also found that the DCs producing retinoic acid exhibit a DC1 phenotype. This shift in the CLN dendritic cell population correlated with increased number of CCR9+ a4b7+ CD8+ T cells. Together this data shows that congenital CMV reprograms the CLN to induce a brain-homing phenotype in CD8+ T cells that is critical to control of viral replication. This work is significant because it provides the first identified mechanism for T cell recruitment into the brain during congenital viral infection. These findings challenge the previous dogma that retinoic acid/CCR9 signaling axis was a uniquely gut-tropic phenomenon.

Poster No. 16

Identification and Characterization of Conformer Specific MHC Class II Anti- I-A^d Monoclonal Antibodies

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The major histocompatibility complex (MHC) is an important part of the immune system. MHC class II molecules are expressed on dendritic cells and macrophages and are involved in initial activation of T cells. MHC class II molecules are also responsible for B cells presenting exogenous antigen-derived peptide to CD4 T cells. This function is essential for T and B cell activation and therefore a robust immune response. The MHC class II molecule is a heterodimer composed of a (alpha) and b (beta) chains. It has been shown that MHC class II molecules exist in two distinct conformations known as M1 and M2-paired class II. Each conformer differs in their pairing of transmembrane (TM) domain sequences. The TM domain of the a (alpha) chain contains two GxxxG dimerization motifs. In contrast, the b (beta) chain only contains one GxxxG motif which can pair with either a (alpha) chain motif forming the two different MHC class II conformers. To determine the roles of the two MHC class II conformers in the immune response, conformer specific monoclonal antibodies (mAbs) are a key tool. Previously, the lab identified and characterized mAbs distinguishing M1-paired vs all MHC class II for mouse I-A^k and human HLA-DR. Using the anti-I-A^k conformer specific mAbs, the M1 and M2 paired conformers were shown to have unique immunological and biological properties. Preliminary studies suggest a well-known anti-I-A^d mAb is specific for the M1-paired conformer, whereas a second anti-I-A^d mAb appears to be pan-reactive. I-A^d class II is expressed in multiple commonly used mouse models such as BALB/c. Identifying mAbs for M1 or M2-paired I-A^d will allow further study of how activation/blockade of each conformer impacts the immune response.

Poster No. 17

Post-operative delirium: Elucidating the role of High mobility group box 1 (HMGB1) signaling in surgery-induced neuroinflammation

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The blood-brain barrier (BBB) is an essential multicellular, neuroprotective structure that prevents the extravasation of peripherally circulating immune cells such as T-cells, neutrophils, and monocyte-derived macrophages. However, sterile surgery can activate the innate immune system and lead to a plethora of pathologic immune signaling that compromises the integrity of the blood-brain barrier and can elicit delirium, a neurocognitive disorder associated with patient confusion and disorientation. Furthermore, age (over 65) and genetic predisposition to neurodegenerative diseases are the greatest risk factors for developing postoperative delirium (POD). High mobility group box 1 (HMGB1) is a prominent amplifier of inflammation – when released extracellularly - it acts as a DAMP modulating inflammation from the site of injury to the BBB and the brain's resident immune cells (microglia). Our lab has previously demonstrated that microvascular endothelial cells of the BBB increase vascular cell adhesion molecule type 1 (VCAM-1) expression and neutrophil extracellular traps (NET) formation (Miller-Rhodes et al., 2022). We are interested in investigating the extent to which endothelial cells are affected by HMGB1 and how HMGB1 expression increases after orthopedic surgery in murine models.

An immortalized microvascular endothelial cell line (bEND3) was used to conduct in-vitro assays with a 24-hour incubation of HMGB1. The Transendothelial electrical resistance (TEER), a measurement of the barrier integrity, was tested before incubation and 24 hours after incubation. An ICC analysis was also done to detect VCAM-1 and tight junction protein expression. We also performed tibia fractures on 12-week-old C57BL6 mice. We collected brain samples for IHC analysis of HMGB1 and Western blot and qt-PCR analysis.

We anticipate that exposure to HMGB1, akin to a systemic increase in plasma levels, will negatively impact endothelial cells by exacerbating neuroinflammatory markers and decreasing the expression of tight junction proteins. By investigating HMGB1 signaling in degrading the BBB, we can begin targeting and antagonizing this pathway as a neuroprotective therapy for postoperative delirium. Other downstream signaling pathways of HMGB1 (TLR4, TLR2, and RAGE) will also be upregulated in Western blot analysis and qt-PCR.

Post-operative delirium is the most prevalent complication for elderly adults. By investigating HMGB1 signaling in disrupting the BBB, we can begin targeting and antagonizing this pathway as a putative neuroprotective therapy for postoperative delirium.

Poster No. 18

Stimulating the Type I Interferon Pathway to Enhance the Efficacy of Short Course Radiotherapy in Rectal Cancer

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Colorectal Cancer (CRC) is a devastating malignancy that ranks as the third leading cause of cancer related deaths and the third highest rate of cancer diagnosis each year in the United States. Among CRC cases, rectal cancer (RC) makes up one third of cases, with over 40,000 people in the US diagnosed each year. Surgical resection is a treatment option for RC, however it can greatly reduce patients' quality of life. As a result, short course radiation therapy (SCRT) is being investigated as an alternative method to treat RC. SCRT can significantly reduce tumor burden in a subset of patients, however most patients' tumors do not respond to radiotherapy (RT), and this divide is poorly understood. To investigate this phenomenon, we developed an orthotopic murine model of RC that recapitulates human rectal tumors. In this model, administration of clinically relevant SCRT results in some mice that exhibit a reduction of tumor burden (responders), and a group of mice whose tumor respond poorly (nonresponders). RNA-sequencing of intratumoral cell populations revealed an upregulation of the Type I Interferon (IFN) signaling pathway in responder tumors when compared to nonresponders. Type I IFNs have emerged as essential cytokines that mediate the antitumor immune response elicited by the damage-associated molecular patterns (DAMPs) produced in response to RT. Preliminary data has confirmed that responder tumors had higher concentrations of intratumoral Type I IFN protein that were maintained throughout treatment. Blockade of the Type I IFN receptors and the cGAS/STING pathway completely abrogated the responder phenotype further emphasizing the importance of these factors in dictating the responder/nonresponder divide. We translated these findings to a therapeutic intervention where tumors were treated concurrently with SCRT and a cGAS-STING agonist designed to augment Type I IFN concentrations. Combination treatment resulted in a higher percentage of responder tumors and markedly improved treatment efficacy. We have expanded on these studies and tested the responder/nonresponder divide using a novel colorectal cancer cell line (KAP cells) that harbors the three most clinically relevant mutations (KRAS, APC, and p53). Similarly, SCRT of KAP cells also produces a responder/nonresponder divide. Collectively, our data has identified intratumoral Type I IFN and the cGAS-STING pathway as driving factors that govern the responder/nonresponder divide to SCRT in RC. More importantly, interventions that target these particular pathways are likely to lead to a higher percentage of patients that respond to SCRT.

Poster No. 19

Differential Immunoregulatory Roles of Surfactant Protein A Variants in SARS-CoV-2 Infected Humanized Transgenic Mice

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GWAS studies have demonstrated a link between multiple genetic loci, including surfactant protein gene locus with COVID-19 severity. Human surfactant protein A (hSP-A) is a collectin expressed in the lung and other mucosal tissues and plays a critical role in innate immunity. HSP-A gene is highly polymorphic, including several genetic variants, i.e., SP-A1 (variants $6A^2$, $6A^4$), and SP-A2 (variants $1A^0$, $1A^3$). Recently, we found that these SP-A variants differentially interact with SARS-CoV-2 spike protein and inhibit viral infectivity *in vitro*. This study further assessed the antiviral and immunoregulatory mechanisms of SP-A variants *in vivo*.

Four double-humanized transgenic mouse lines with hACE2 and hSP-A variant (hACE2/$6A^2$, hACE2/$6A^4$, hACE2/$1A^0$, and hACE2/$1A^3$), one SP-A deficient (hACE2/SP-A KO) and one K18 (hACE2/mouse SP-A) mice were challenged intranasally with SARS-CoV-2 (Delta, 10^3 PFU/mouse) or mock-challenged with saline. The mice were monitored for 10 days or sacrificed 6 days post-infection, then various histological, cellular, molecular, and serological analyses were performed.

The results showed that infected hACE2/SP-A KO mice had more weight loss and mortality compared to those with human or mouse SP-A ($P<0.05$). Severe organ injuries (lung, kidney, brain, intestine) were observed in infected mice with more severity in hACE2/SP-A KO mice compared to humanized SP-A mice. hACE2/$1A^0$ and hACE2/$6A^4$ mice showed less severe lung and kidney injuries compared to other mouse lines. Viral load in the lung of SP-A KO, hACE2/$1A^3$, and hACE2/$6A^4$ mice was remarkably higher than in other mouse lines ($p<0.01$). Transcriptomics studies revealed an upregulation in innate and adaptive immune genes such as *MyD88*, *IL17A*, and *Mx1* in the lung of infected SP-A KO mice compared to infected mice with SP-A transgenes. However, *Mapk1* was significantly downregulated in infected hACE2/$6A^2$ vs hACE2/$1A^0$ mice ($P<0.05$). Ingenuity pathway analysis of differentially expressed transcripts (>2.0-fold change, $p<0.05$) revealed the involvement of several biological pathways in the lung of infected mice, including pathogen-induced cytokine storm, NOD1/2, and Trem1 signaling pathways. IL22 and 4-1BB signaling in T lymphocytes were the top canonical pathways identified in infected hACE2/$6A^2$ vs hACE2/$1A^0$ mouse lines. Furthermore, levels of cytokines and chemokines such as TNFα, IFN-γ, IL-10, IL-12, and RANTES were remarkably increased in the sera of hACE2/SP-A KO, hACE2/$1A^0$, and hACE2/$6A^4$ vs hACE2/$6A^2$ and hACE2/$1A^3$ lines.

These findings demonstrate that human SP-A variants differentially attenuate SARS-CoV-2-induced tissue injury by differentially inhibiting viral load and regulating signaling pathways *in vivo*. The data support efforts at producing personalized surfactant-based therapies for COVID-19 patients.

Poster No. 20

Neuroinflammation and neurovascular injury in SARS-CoV-2 infected mouse model

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Background: Although the SARS-CoV-2 virus primarily infects the respiratory system, there are reports of neuropathology during and post-infection. The short-term neurological manifestations include headache, anosmia, and brain fog. In the long term, the infection results in Alzheimer's disease-like phenotypes in individuals without the disease prior to SARS-CoV-2 infection and rapid progression in people who have Alzheimer's disease before infection. Some mural cells known as pericytes wrap around capillaries in the brain. These cells have various functions, including maintaining the blood-brain barrier (BBB) and angiogenesis. The expression of FLI-1 transcription factor increases during the development of Alzheimer's disease, particularly in the hippocampus, while the number of pericytes decreases. The goal of the study is to examine how SARS-CoV-2 infection causes the development of neurological abnormalities.

Methods: Humanized transgenic mice with hACE2 were infected intranasally with the SARS -CoV-2 (Delta) variant with 10^3 PFU/mouse or saline (control). Brain tissues from infected mice were harvested and fixed 6 days post-infection for analysis. H&E staining sections were used for brain inflammatory analysis. Neurovascular damages, specific microvascular cells (Pericytes), and one relevant transcriptional factor (FLI-1) in the brain tissues were examined by histological and specific-antibody immune analyses. Additionally, primary neurons/microglia from 2-day mice pups were used for studying the immune response to SARS-CoV-2 Spike or Envelope protein stimulation. It was statistically significant when $p<0.05$ by t-test or ANOVA.

Results: The results show remarkable brain inflammation and neurovascular damage in the SARS-CoV-2 infected mice compared to controls. SARS-CoV-2 virus neurotropism was detected by the presence of viral N-protein in the brain tissues of infected mice but not in control mice. Immune staining with FLI-1 antibody demonstrated an increased level (by 14-35%) of Fli-1 in the brain tissues of infected mice compared to the uninfected mice, suggesting the potential of these mice to develop Alzheimer's-like phenotype in the future. Microvascular damages in the brain tissues, especially in the hippocampus, were analyzed by specific biomarker CD13 (pericytes and other cells), indicating microvascular injury in the infected mice. Furthermore, in vitro studies with primary neurons/microglia from the brain of mouse pups indicate that viral S and E proteins stimulated the production of IL-6.

Conclusion: These results indicated that SARS-CoV-2 infection causes inflammation and neurovascular (BBB) damage in the brain and upregulates the expression of Alzheimer's disease-relevant transcriptional factor (FLI-1) which has a high potential to induce the death of pericytes, and the development of Alzheimer's disease and other neuropathologic conditions.

Poster No. 21

Neonatal CD8+ T cells resist terminal exhaustion during chronic infection

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While recent advances in our understanding of CD8+ T cell biology have led to revolutionary immunotherapies for cancer and chronic viruses, T cell exhaustion remains a major barrier to therapeutic efficacy. CD8+ T cell exhaustion is the hierarchical loss in proliferation, cytokine production, and effector function of CD8+ T cells after chronic antigen exposure. An important and unanswered question is why do some CD8+ T cells become more exhausted than others. Our lab previously showed that heterogeneity in the CD8+ T cell response to acute pathogens is linked to their developmental origins. Specifically, adult T cells (derived from bone marrow stem cells) become memory precursors, while neonatal cells (derived from fetal progenitor cells) become short-lived effectors. Thus, we hypothesized that CD8+ T cell fate during chronic infection, including degree of T cell exhaustion, is also linked to their initial time of production. To test this hypothesis, we transferred P14 transgenic adult and neonatal CD8+ T cells into congenically distinct recipient mice and compared their responses to a chronic virus (LCMV clone 13) using high-parameter flow cytometry, functional assays, and RNA sequencing. We found that early in chronic infection, neonatal cells preferentially became highly functional effector cells expressing KLRG1 and CX3CR1, while adult cells differentiated into exhausted cells. Interestingly, neonatal cells' effector skew corresponded with enhanced expansion within the host and migration into tissues early in infection. Importantly, neonatal CD8+ T cells also conferred greater protection from viral replication early in chronic infection. Late in infection, neonatal cells produced more interferon gamma and TNF alpha than their adult counterparts. Neonatal cells also retained a less terminally exhausted phenotype than adult cells, which expressed high levels of PD-1 and Tim3. To understand whether these differences exist in other contexts of chronic T cell stimulation, we are currently comparing the adult and neonatal CD8+ T cell responses to tumors. Together, our data suggests that the developmental origin of CD8+ T cells plays a deterministic role in their fates during chronic infection.

Poster No. 22

Investigating the role of HE4-mediated immunosuppression in ovarian cancer

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Epithelial ovarian cancer (EOC) is the leading cause of death among patients with gynecologic malignancies. EOC is typically responsive to first-line chemotherapy, but exhibits high recurrence rates and eventually becomes resistant. Although the importance of the immune system has long been recognized in ovarian cancer outcomes, immunotherapies have poor response rates in EOC compared to other gynecologic cancers. Therefore, a better understanding of the immune response to EOC is needed to develop improved treatments.

HE4 is a secretory glycoprotein that is produced in normal respiratory tract and genital epithelium and is overexpressed in most serous ovarian cancers. HE4 is a member of the whey acidic protein (WAP) family of immunomodulators that typically function as serine protease inhibitors and have shown antimicrobial and wound healing effects. HE4 is used as a serum biomarker for EOC, and elevated levels are associated with a poor prognosis. It has also been shown to be upregulated in the presence of persistent immune activation and inflammation in benign tissues, however, the specific function of HE4 in EOC remains unclear.

Data from our lab has shown that patient tissue with HE4 high expressing tumors have fewer infiltrating CD8+ T cells and higher tumoral PD-L1 expression compared to HE4 low expressing tumors. In addition, rats with tumors overexpressing HE4 have a more aggressive cancer phenotype with dramatic changes in the tumor microenvironment (TME) characterized by more suppressive macrophages (M2), increased CCL2 and IL-10 production, and reduced activated cytotoxic T cells. Therefore, we hypothesize that HE4 promotes an immunosuppressive TME through the CCL2/CCR2 axis, promoting the recruitment of suppressive macrophages and inhibiting T cell responses at the tumor site. To test this hypothesis, we will use a new mouse model of EOC that closely recapitulates the human EOC TME to dissect the function of HE4. Preliminary data has shown that mice with ovarian cancer treated with an experimental small molecule inhibitor of HE4 show a repolarization of monocyte populations from an anti-inflammatory phenotype to a pro-inflammatory phenotype, indicating a potential therapeutic avenue for inhibiting HE4 in ovarian cancer. Developing a better understanding of the tumor immune microenvironment and determining the precise role of HE4 will be essential in developing effective immunotherapies for EOC in the future.

Poster No. 23

IFN-gamma induced microglial re-programming

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IFN-gamma (IFN-g) plays a crucial role in defending against intracellular pathogens, including *Toxoplasma gondii*, an intracellular parasite that chronically infects one-third of the world's population. Our previous studies have demonstrated that IFN-g is a major effector cytokine, regulating multiple host defense pathways, and triggering immunopathological responses characterized by the loss of epithelial and myeloid cells at the infection site. Using multicolor flow cytometry and imaging experiments, we found that the acute response to *T. gondii* leads to a rapid depletion of brain-resident macrophages, specifically microglia. Similarly, we also observed loss of the other tissue-resident macrophage populations including intestinal macrophages, liver Kupffer cells, and large peritoneal macrophages.

Our experiments revealed that early IFN-g production induces a profound response in the brain prior to parasite invasion into the tissue. Analyzing reporter mice that allow tracking of cells responding to IFN-g via detection of CXCL10, we observed a significant response in microglial cells to IFN-g. Additionally, IFN-g was found to cause a notable reduction in microglial populations during the acute stage of infection, independent of the parasite's presence in the brain. Flow cytometric analysis further confirmed the disappearance of microglia during the acute immune response to the parasite. Overall, our data revealed a significant decrease in the microglial population in the brain during the innate phase of *T. gondii* infection. Consequently, we propose further investigation into the mechanism and cellular dynamics of IFN-g-mediated effects on microglia.

Poster No. 24

Discovery of TIGIT inhibitors by phage display

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TIGIT is an emerging immune checkpoint receptor involved in both innate and adaptive immune responses due to its expression on NK cells, cytotoxic T cells and regulatory T cells. By competing with the co-stimulatory receptor CD226/DNAM-1 for binding to CD155/PVR, TIGIT transduces inhibitory signals and suppresses the immune response. Therefore, we sought to discover TIGIT inhibitors for improving tumor immunotherapy. Through directional cloning, bacterial transformation, and phage amplification techniques, we generated a novel genetically encoded, phage-displayed cyclic peptide library comprised of more than a million peptide sequences. Each peptide is predicted to form a cyclic structure via disulfide bonds on the side chain of cysteine residues. We conducted three rounds of phage display panning against the recombinant TIGIT IgV domain that enriched high-affinity peptides and identified promising TIGIT binding peptides. Phage ELISA showed binding of phage-presented peptides to recombinant TIGIT IgV and competitive ELISA evaluated the ability of the peptides to disrupt interactions between TIGIT and PVR. Cellular assays assessed the function of the peptides to enhance cytotoxic T cell activity. This work validated the efficiency of the phage-displayed cyclic peptide library for discovery of ligands targeting protein receptors and led to identification of novel TIGIT inhibitors.

Poster No. 25

Dual Armored CAR T Cell Therapy for Pediatric Sarcomas

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Chimeric antigen receptor (CAR) T cell therapy has limited efficacy in solid tumors despite tremendous success in hematological malignancies. Major obstacles include heterogenous expression of tumor associated antigens and the immunosuppressive tumor microenvironment, resulting in disease progression from target antigen escape and impaired CAR T cell function. Strategies to overcome these barriers include activation of endogenous immunity through CAR T cells further modified to release immunomodulatory molecules, referred to as "armored CAR T cells". We have developed novel CARs targeting survivin, which is overexpressed on multiple cancers including sarcomas. Our preliminary data demonstrates in vitro and in vivo cytotoxicity of anti-survivin CAR T cells against murine and human pediatric sarcomas. To further enhance anti-survivin CAR T efficacy, our lab is engineering these CAR T cells to secrete interleukin 18 and express CD40 ligand. Our previous publications demonstrated that IL-18 secreting CAR T cells recruit and activate endogenous T cells which mediate heterogenous antigen tumor clearance. We also showed that CD40L-expressing CAR T cells activate and mature antigen presenting cells which prime and recruit endogenous T cells for improved antitumor response. We will investigate whether IL-18 and CD40L dual armored anti-survivin CAR T cells exhibit superior antitumor efficacy compared to unarmored CAR T cells through unique and complementary pathways, as well as the immune cell types in the tumor microenvironment that mediate the effect. Our work will uncover a novel combination strategy that can improve CAR T cell efficacy for pediatric sarcoma patients.

Poster No. 26

Exploration of the BTN2A1/BTN3A1 interaction by point mutations and HMBPP analogs

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Phospho-antigens initially bind to the intracellular B30.2 domain of the butyrophilin protein BTN3A1, which induces a conformational change in BTN3A1, allowing it to interact with the BTN2A1 protein. Once bound, the extracellular domain of BTN2A1 can engage with the T cell receptor (TCR) protein on the surface of γδ (gamma delta) T cells, leading to the activation of γδ T cells and subsequent target cell lysis. However, certain aspects of BTN2A1 protein interactions remain incompletely understood, such as how the intracellular domain of BTN2A1 interacts with the BTN3A1 protein.

To gain further insights into this process, our research focused on introducing specific amino acid mutations in the intracellular domain of BTN2A1 and synthesizing (*E*)-4-hydroxy-3-methyl-but-2-enyl diphosphate (HMBPP, a major phospho-antigen) analogs. The objective was to assess how these mutations impact the molecular binding ability of BTN2A1 to BTN3A1 (using isothermal titration calorimetry - ITC) and the biological activity of γδ T cell activation (using enzyme-linked immunosorbent assay - ELISA), and how the synthesized HMBPP analogs affect the interaction between BTN2A1 and BTN3A1.

The results revealed that mutations in the C247, C265, and EKE282 residues of the intracellular domain of BTN2A1 had no effect on its activity. However, mutations in the EELRWR316-321 and RTFLH322-326 residues, especially L318G and L325G, significantly abrogated subsequent γδ T cell activation. Additionally, we designed and synthesized five HMBPP analogs. These HMBPP analogs were found to bind to the B30.2 region of BTN3A1 intracellular domain. However, despite this binding, the BTN3A1 protein was unable to interact with the BTN2A1 protein as well as fully activating γδ T cell.

Overall, our study identified critical amino acid residues involved in the interaction between BTN2A1 and BTN3A1. Furthermore, it revealed the potential of certain phospho-antigen analogs to act as inhibitors, blocking the interaction between BTN2A1 and BTN3A1. These findings contribute to a deeper understanding of the activation of γδ T cells and may have implications for the development of therapeutic agents targeting this pathway.

Poster No. 27

CasL regulates CD4$^+$ T cell migration by restricting membrane blebbing

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The ability of T cells to migrate into and within lymphoid and peripheral tissues is crucial for adaptive immunity. Integrin signaling is known to be an important factor controlling immune cell migration, but the signaling events that regulate cytoskeletal changes during T cell migration remain poorly defined. Here, we have identified the scaffold protein, CasL, as a central regulator of integrin signaling and T cell migration. Primary mouse T cells lacking CasL have severe defects in actin polymerization and overall migration on integrin ligand ICAM-1 coated surfaces. The knockout T cells were slower, less directional, and displayed multiple abhorrent protrusions, suggesting an essential role for CasL in orchestrating cytoskeletal dynamics. Interestingly, closer inspection of migrating CasL KO T cells revealed the high prevalence of blebs; membrane protrusions indicative of either a weakened cortical actin cytoskeleton or dysregulated actomyosin contractility. To test these distinct possibilities, we performed a biochemical analysis of cytoskeletal signaling pathways and found that depletion of CasL did not alter cortex-membrane connections but did result in increased actomyosin contraction through regulation of myosin light chain kinase. These data suggest that CasL normally functions to limit myosin-based contraction in migrating T cells. Importantly, treatment of CasL KO cells with an inhibitor to Rho-associated protein kinase (a master regulator of actomyosin contraction) completely reversed their migratory defects. These data strongly support a role for CasL as a negative regulator of actomyosin contraction during T cell migration and reveal a novel signaling mechanism that could be exploited for future cell-based therapeutics.

Poster No. 28

The roles of CD49a and CD103 on tissue resident memory (T_{RM}) CD8 T cell function and retention in the lungs and airways

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The population is experiencing a resurgence of Influenza related infections and hospitalizations after the COVID-19 pandemic. In order to formulate better vaccines, it's imperative to understand the mechanisms that can reduce disease burden. Tissue-resident memory (T_{RM}) CD8 T cells in the airways and lungs are among the first line of defense against viral reinfection. The ability to provide surveillance at the primary infection site distinguishes T_{RM} from other CD8 memory T cells.

The expression of integrins CD49a and CD103 identifies subsets unique to T_{RM} in the pulmonary tissues compared to the generalized CD69 T_{RM} surface marker. CD103 binds to E-cadherin on epithelial cells. CD49a binds to collagen IV in the basement membrane of the lungs and can be detected on CD8 T cells as early as 6-8 days post-infection. CD49a has a role in T_{RM} adhesion, lung and airway retention, and tissue locomotion. Anti-CD49a antibody blockade administered before secondary infection reduces T_{RM} in the lungs and leads to a loss of virus-specific CD8 T cells in pulmonary tissues.

CD49a knockout (KO) or CD49a antibody blockade do not specifically target CD8 T cells and could lead to outcomes that aren't unique to integrin function on CD8 T cells. To target this mechanism to CD49a on T_{RM}, we developed an adoptive transfer and influenza-infection model, where CD49a is specifically-deleted in CD8 T cells (conditional knockout: iCD8 *ItgA1* KO) by treating the animals with Tamoxifen for 5-consecutive days. We tested how integrin expression impacts cell location by surveying the lungs, airways, and secondary lymphoid organs. iCD8 *ItgA1* KO mice were treated with Tamoxifen beginning on day 32 post-infection to trigger loss of CD49a. Flow cytometric analysis showed that iCD8 *ItgA1* KO mice had reduced CD49a+ memory CD8 T cells in the lungs, suggesting the model works as expected. In the future, we will investigate effector function by stimulating and intracellularly staining iCD8 I*tgA1* KO cells for flow cytometric analysis.

T_{RM} micro-position is important for mediating antiviral responses. We observed virus-specific CD8 T cells in proximity with trachea submucosal glands (tSMGs), which have high E-Cadherin expression. Former lab members showed that Influenza-virus replication can occur within the tSMGs. We hypothesize that integrins mediate micro-positioning of the T_{RM} to allow the cells to sample the environment for viral antigen. We predict that T_{RM} are positioned relative to Collagen IV, E-cadherin, or both structures based on the integrin expression on the T_{RM}. eCFP OT-I T cells will be adoptively-transferred into B6 recipients. The trachea will be cryosectioned, fluorescently-stained for integrin (CD49a and CD103) and ligand (Collagen IV and E-cadherin) expression to image with the confocal microscope. This experiment will address the distribution of T_{RM} relative to integrin expression and ligand interactions.

These experiments will help define the function of CD49a and CD103 in local retention, positioning, and antiviral immunity.

Poster No. 29

Investigating the Role of *Bifidobacterium longum ssp. infantis* in Maintaining Intestinal Epithelial Cell Barrier Integrity

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Background: Food Allergy (FA) is a potentially life-threatening condition that has become increasingly prevalent and affects ~8% of children in the United States. We recently studied Old Order Mennonites (OOM) who maintain an agrarian lifestyle protected against allergic diseases. We found that the OOM infant gut microbiome reveals a high colonization rate of *Bifidobacterium longum ssp. infantis* (*B. infantis*), probiotic organisms suggested to play a role in immune response and allergy development. To better understand their protective characteristics, we investigated how two *B. infantis* strains affect intestinal permeability.

Methods: Caco-2 epithelial cell monolayers were treated in triplicate with supernatants from OOM infant-isolated *B. infantis* strains UMA0003 and UMA0005, with and without lipopolysaccharide (LPS), a toll-like receptor 4 (TLR4) agonist known to induce intestinal barrier dysfunction. During incubation, tight junction permeability was assessed using Trans-Epithelial Electrical Resistance (TEER) values, which were measured at 0 and 4 hours. Immunofluorescence (IF) staining and microscopy were used to visualize the expression of tight junction protein ZO-1.

Results: The average change in TEER over 4 hours was positive for cells treated with Lacto-N-tetraose (LNT) and Caco-2 growth medium and comparable to TEER of cells treated with *B. infantis* UMA0003 and UMA0005. Neither strain mitigated the disruptive effect of LPS. Interestingly, immunofluorescence (IF) staining and microscopy revealed that cells treated with *B. infantis* supernatant appeared larger and more regularly proliferated.

Conclusions: Preliminary data does not suggest that *B. infantis* strains UMA0003 and UMA0005 have an effect on the barrier integrity of intestinal epithelial cells. In future experiments, we will treat cells for longer periods, up to 48 hours. Additional microscopy and ZO-1 staining will be conducted to confirm gross differences in cell characteristics after treatment with *B. infantis* and other *Bifidobacterium* strains.

Keywords: Caco-2 cells, *Bifidobacterium longum ssp. infantis* (*B. infantis*), Lipopolysaccharide (LPS), intestinal permeability, barrier integrity, allergy, microbiome.

Poster No. 30

Elucidating the Mechanism of CCL3 Driven Bone Marrow Dysfunction during Acute Myelogenous Leukemia

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Acute Myeloid Leukemia (AML) is an aggressive adult blood cancer, and is characterized by a dysbiosis in the myeloid and lymphoid progenitors' differentiation leading to an accumulation of immature cells. This leads to a loss in normal hematopoiesis and overall disruption of the bone marrow microenvironment (BMME).

Studies show that C-C Motif Chemokine Ligand 3 (CCL3), which signals through two G-Protein coupled receptors, CCR1 and CCR5, has been associated with hematopoietic malignancies and BMME dysfunction [Staversky et al., 2018]. Furthermore, our initial research shows that CCL3 released by leukemic cells promotes their migration and proliferation [Ackun-Farmmer et al., 2021]. Our research in blocking CCL3 signaling using inhibitors delivered via bone-targeted nanoparticles showed a partial reduction in leukemia in blast-crisis Chronic Myelogenous Leukemias (bcCML) murine model, a clinically relevant model of AML [Ackun-Farmmer et al., 2021].

Thus, to understand the mechanism of CCL3-driven dysfunction in the BMME, we are investigating the effect of globally knocking out CCL3 ligand receptors, CCR1 and CCR5, in single knockouts of either receptors and double knockout (dKO) of both receptors as compared to wild type (WT) C57/Bl6J and bcCML mice. We are longitudinally characterizing the hematopoietic populations of healthy and leukemic dKO mice in comparison to WT at regular intervals. Our initial analysis of a complete blood counts (CBCs) showed no significant phenotypic differences between the healthy dKO mice and WT mice. However, initial CBC analyses of leukemic dKO mice show a significant increase in the erythroid populations, namely the RBC (P value=0.019), hemoglobin (P value=0.018), and hematocrit count (P value=0.02), as compared to the WT leukemic mice where these populations showed depletion. This may be due to lack of CCR1/5 receptors in the dKO model which do not respond to leukemic signaling. Furthermore, initial analysis of the myeloid and lymphoid precursor populations of healthy dKO vs. WT did not show any significant differences in the progression of the hematopoietic system. Therefore, CCR1/5 receptors may not be involved in regulating the hematopoietic balance, which suggests that they may be ideal targets for an AML-specific therapeutic.

Further, we aim to continue to decipher the phenotypic and functional capacity of the BMME components which include the hematopoietic stem and progenitors cells (HSPC), mesenchymal stem cells (MSC), the osteoblasts, and the macrophages in healthy and leukemic WT vs. dKO mice. We also aim to generate a CCR1/5 knockout leukemia cells and test their capacity in initiating leukemia.

Poster No. 31

Investigation of Influenza-Reactive Memory B Cells in the Human Spleen

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B cell mediated immunity to viruses is a function of pre-existing antibodies (Abs) with antiviral activity and memory B cells (MBCs), which respond to re-exposure with rapid, vigorous, and high affinity Ab production. After formation in responding lymphoid tissues, MBCs enter the circulation and disperse to lymphoid tissues throughout the body, including the spleen. Due to its vast storage capacity, the human spleen is regarded as key MBC reservoir. Recent studies suggest that the spleen is also an "archive" of all MBC clones generated in B cell responses. Furthermore, it is suggested that a subset of splenic MBCs receive signals in a marginal zone niche that increases responsiveness to activating signals and promotes longevity. Our goal was to investigate the splenic archive hypothesis using the hemagglutinin (HA) of influenza virus as a model antigen. Adults typically carry a large pool of HA-reactive MBCs that have been modified by multiple exposures to HA variants over many years. In addition, an individual's MBCs pool often carries a lasting imprint of significant influenza infection in early life. The size of HA-reactive MBC populations in stored human splenocytes was determined by in vitro MBC stimulation to induce Ab-secreting cell formation, with multiplex analysis of secreted Ab binding to a broad range of HAs. To characterize MBC subsets, we developed a full spectrum flow cytometry panel with HA probes for deep immunophenotyping of HA-reactive MBCs. Findings to date identify IgG MBC populations in the spleen with collectively broad HA reactivity and subsets reflecting functional differences. Our analysis adds to the concept of the spleen as an important MBC reservoir and identifies biomarkers indicative of MBC function and durability.

Poster No. 32

Combination Adjuvants and Antigen Provide Heterosubtypic Protection against Lethal Influenza Infection

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Influenza A virus (IAV) is a negative strand RNA virus that undergoes antigenic drift and shift, leading to serologically distinct seasonal and pandemic strains that can cause tens of thousands of deaths annually in the US. The wide variety of hemagglutinin (HA) and neuraminidase (NA) proteins necessitates yearly reformulation of the vaccine to protect against new, circulating seasonal strains. Current IAV vaccines do not contain adjuvants, however, immune modulators such as pattern recognition receptor (PRR) agonists may enhance antigen presentation to T cells and provide broader protection against IAV strains that differ in HA and NA outer coat proteins. We set out to investigate whether TLR4 (lipopolysaccharide) and TLR3 (poly IC) agonists in combination with HA (H3N2) and nucleoprotein (NP) or NP and matrix (M1) proteins could provide protection against lethal challenge with mismatched IAV strain H1N1. Mice given H3 and NP with LPS + pIC in a prime/boost strategy demonstrated 80% survival after heterosubtypic H1N1 challenge while H3 + NP in PBS showed 20% survival. Similar results were seen using LPS + pIC in combination with NP and M1 protein with 90% survival. High endpoint titers of anti-NP antibodies that cross reacted with PR8 H1N1 virus, correlated with heterosubtypic protection suggesting a role for non-neutralizing antibodies in promoting survival. Further, LPS and pIC increased migratory monocyte DC in the lymph node in vivo, coupled with early upregulation of T cell marker CD69 when administered intramuscularly. Similarly, intranasal administration of LPS and pIC with protein antigen induced high peptide specific IFN-gamma production in CD4 T cells and > 90% survival indicating a role for T cells and mucosal immunity. This combination strategy may provide increased efficacy over current IAV vaccines by inducing non-neutralizing antibodies and enhanced T cell responses to internal proteins.

Poster No. 33

The emergence of SARS-CoV-2 lineages and associated saliva antibody responses among asymptomatic individuals in a large university community

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SARS-CoV-2 (CoV2) infected, asymptomatic individuals are an important contributor to COVID transmission. CoV2-specific immunoglobulin (Ig)—as generated by the immune system following infection or vaccination—has helped limit CoV2 transmission from asymptomatic individuals to susceptible populations (e.g. elderly). Here, we describe the relationships between COVID incidence and CoV2 lineage, viral load, saliva Ig levels (CoV2-specific IgM, IgA and IgG), and ACE2 binding inhibition capacity in asymptomatic individuals between Jan 2021 and May 2022. These data were generated as part of a large university COVID monitoring program in Ohio, USA, and demonstrate that COVID incidence among asymptomatic individuals occurred in waves which mirrored those in surrounding regions, with saliva CoV2 viral loads becoming progressively higher in our community until vaccine mandates were established. Among the unvaccinated, infection with each CoV2 lineage (pre-Omicron) resulted in saliva Spike-specific IgM, IgA, and IgG responses, the latter increasing significantly post-infection and being more pronounced than N-specific IgG responses. Vaccination resulted in significantly higher Spike-specific IgG levels compared to unvaccinated infected individuals, and uninfected vaccinees' saliva was more capable of inhibiting Spike function. Vaccinees with breakthrough Delta infections had Spike-specific IgG levels comparable to those of uninfected vaccinees; however, their ability to inhibit Spike binding was diminished. These data are consistent with COVID vaccines having achieved hoped-for effects in our community, including the generation of mucosal antibodies that inhibit Spike and lower community viral loads, and suggest breakthrough Delta infections were not due to an absence of vaccine-elicited Ig, but instead limited Spike binding activity in the face of high community viral loads.

Poster No. 34

Comprehensive analysis of thymic selection throughout development reveals a wave of innate-like CD8+ T cells produced in early life.

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$CD8^+$ T cells developed in early life exhibit distinct phenotypic and functional characteristics compared to those generated in adulthood. However, the specific timing and mechanisms by which these differences arise during ontogeny remain poorly understood. To address this gap in knowledge, we conducted a comprehensive analysis of thymic selection in mice at various stages of life. Interestingly, our research unveiled the presence of a distinct wave of self-reactive $CD8^+$ T cells with higher affinity for self-peptides that are produced in mice at one week of age. To gain further insights into the kinetics of thymic selection during early life, we conducted measurements of clonal deletion at the population level. Surprisingly, we found lower proportions of thymocyte death in early life despite elevated levels of self-reactivity. This wave of thymocytes at one week undergo clonal deletion at lower rates. Moreover, we observed that this wave of mature $CD8^+$ T cells in the thymus expresses high levels of Eomes, the master regulator transcription factor of virtual-memory phenotype acquisition in the periphery. To understand why this innate-like subset arises during the neonatal period, we are performing intrathymic injections to compare how thymic selection is altered in CD8+ T cells derived from fetal and adult progenitors. These studies are crucial as they normalize the thymic environment and offer key insights into how developmentally-related changes in T cell production impact the composition of the CD8+ T cell compartment.

Poster No. 35

Amphiregulin Treatment Promotes Satellite Cell and Fibro-adipogenic Progenitor Functions during Chronic *T. gondii* Infection

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Toxoplasma gondii is an obligate intracellular parasite that can cause chronic infection and is able to infect any nucleated cell from warm blooded vertebrates, including mice, an important component of the life cycle. While many existing studies of *T. gondii* involve complications in the central nervous system, like Toxoplasmic encephalitis, there are gaps in understanding how chronic infection impacts skeletal muscle in the context of repair mechanisms. Our previous studies have shown that *T. gondii* leads to chronic infection, myositis, and decreased muscle function in mice. We also found that treatment with the cytokine Amphiregulin (Areg), a ligand of the epidermal growth factor receptor (EGFR), improved muscle function and increased expression of various myogenic factors. What remains unexplored is identifying the target cell of Areg within chronically infected muscle that aids in muscle wound repair. Our study aims to determine how Areg treatment affects stem cell presence and activity found within the muscle, specifically in muscle satellite cells (MuSCs) and fibro-adipogenic progenitors (FAPs). MuSCs are stem cells that express EGFR and differentiate to fuse into new muscle fibers. FAPs are a population of mesenchymal stem cells found in the muscle that also express EGFR and are known to aid MuSCs by creating an environment suitable for differentiation. We are addressing this by examining SC and FAPs during chronic *T. gondii* infection and with EGFR-MuSC deficient mice. The findings of our study will expand our understanding of how Areg promotes MuSC and FAP functions during chronic infection and host-pathogen interactions in the context of wound repair.

Poster No. 36

Sarcomatoid Dedifferentiation Promotes Immunogenic Properties in Renal Cell Carcinoma

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Renal cell carcinoma (RCC) encompasses multiple histological subtypes though these pathologically distinct entities are united in their ability to contain dedifferentiated "sarcomatoid" regions. Sarcomatoid RCC (sRCC) is highly aggressive with a median survival of only 10 months. Conventional cancer treatments including chemotherapy and anti-angiogenic agents are not effective against sRCC; however, immune checkpoint blockade (ICB) has recently induced anti-tumor responses against a portion of sRCC cases. There remain fundamental gaps in understanding the biology underlying sRCC responsiveness to ICB that limit optimization of immunotherapy regimens to improve patient outcomes. To identify potential sources of immunogenicity in sRCC, transcriptomic analysis of the Phase III IMmotion151 trial in RCC was performed. Patients with sRCC had increased tumor mutational burden ($p<0.05$) and expression of PD1 ($p<0.05$) compared to non-sarcomatoid RCCs. Tumor mutational burden is a common surrogate for neoantigen production; therefore, we next sought to identify unique antigens expressed in sRCC. Bulk RNA sequencing of 45 sRCC cases was performed. Expression of potentially immunogenic endogenous retrovirus (ERV) constructs in sRCC tumors was investigated using *hervQuant,* a computational pipeline for mapping genomic sequences to ERV base pair segments (Smith et al. *JCI.* 2019). This revealed aberrant expression of ERV constructs within the HERVH, HERVE, and THE superfamilies, among others, in sRCC. Next, single cell RNA sequencing was performed on RCC cell suspensions from six patients, including two with sRCC. CD45- tumor cells were computationally isolated for gene-set enrichment analysis. Compared to non-sRCC, sRCC displayed upregulation of Interferon-Alpha responses, consistent with retrovirus-like construct recognition. Epithelial-to-Mesenchymal Transition (EMT) (Normalized Enrichment Score > 1.5) and Transforming Growth Factor-Beta (TGF-B) (NES > 1.0) pathways were also upregulated in sRCC tumor cells. To determine if EMT (1) can be induced in epithelial RCC cells and (2) is driving antigen generation, RCC cell lines were cultured in media with TGF-β, after which cells were analyzed for known markers of EMT including CD44 – a marker of cancer cell stemness. CD44 expression was increased in TGF-β treated RCC cell lines relative to controls ($p=0.019$ and $p=0.021$, respectively). Upregulation of CD44 expression in sRCC relative to ccRCC was validated in the IMmotion151 and The Cancer Genome Atlas Clear Cell Renal Cell Carcinoma sequencing datasets ($p<0.01$ and $p<0.05$, respectively). Future work will determine if this EMT derivation model promotes antigen expression in RCC and will validate the ability of putative tumor associated antigens to induce T cell responses *in vitro* and *ex vivo*.

Poster No. 37

Major Outer Sheath Proteins of Understudied Oral *Treponema* species dysregulate neutrophil actin dynamics

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Periodontitis (PD) is a chronic inflammatory condition affecting 47% of adults in the US in some form, leading to destruction of the tooth-supporting tissue. Neutrophils play a prominent role in maintaining oral health, yet may be ineffective in controlling the dysbiotic bacterial plaque community during PD of which multiple spirochete species predominate. The major outer sheath protein (Msp) of the well-characterized species *Treponema denticola* impairs neutrophil function by disrupting phosphoinositide signaling and downstream cytoskeleton dynamics. The phosphoinositide species, phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) and phosphatidylinositol 3,4,5-trisphosphate (PIP_3) and their appropriate regulation are particularly important for actin reorganization, regulating at least 15 different actin binding proteins. The understudied species *T. maltophilum* and *T. lecithinolyticum* have outer membrane proteins similar to Msp: MspA and MspTL, respectively. Our objectives were to characterize the effect of MspA and MspTL on neutrophil phosphoinositide signaling, cytoskeleton dynamics and function.

Murine bone marrow neutrophils were pretreated with recombinant MspA or MspTL proteins, followed by exposure to activating stimuli. Phosphoinositide species were quantified by lipid immunofluorescence, and phosphatase activity was measured with malachite green assays. PI(4)P5 Kinase activity was measured by ATP depletion assays. The actin cytoskeleton was visualized by immunofluorescence, and F-actin filament elongation was quantified by actin monomer incorporation assay. *In vivo* chemotaxis towards *E. coli* engineered to express MspA or MspTL, or intact *T. maltophilum* and *T. lecithinolyticum* was assessed using a murine air pouch model of immune cell recruitment.

MspA and MspTL disorder PIP signaling in neutrophils; significantly increasing PIP3 and $PI(4,5)P_2$ levels in unstimulated neutrophils while preventing appropriate production in stimulated neutrophils. However, neither MspA nor MspTL appear to significantly affect the lipid phosphatases PTEN or SHIP1, which are prominently responsible for regulating $PI(4,5)P_2$ levels. MspA and MspTL increase distribution of cortical actin, yet overall decrease the rate of actin incorporation in stimulated neutrophils. Intact *T. maltophilum* and *T. lecithinolyticum* and *E. coli* expressing MspA or MspTL delay neutrophil recruitment *in vivo*.

Surface proteins from *Treponema maltophilum* (MspA) and *Treponema lecithinolyticum* (MspTL) disrupt phosphoinositide signaling, which leads to dysregulation of the actin cytoskeleton and delayed neutrophil recruitment. Overall, disruption of protective neutrophil functions may contribute to bacterial survival in the oral cavity during periodontitis development and progression.

Poster No. 38

Human Endogenous Retroviruses as Immunogenic Targets in Irradiated Tumors: Have you HERV?

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Renal cell carcinoma (RCC) is relatively sensitive to immunotherapy yet has low tumor mutational burden and predicted neoantigen expression. Given patient responsiveness to immunotherapy and high levels of immune activity in RCC, the immunogenic targets here are likely yet to be identified. One potential source of such antigens are human endogenous retroviruses (HERVs), elements of DNA originating from ancestral viruses that are conserved in the human genome. Recent work has demonstrated a correlation between novel HERV expression and response to immune checkpoint blockade in RCC (Smith et al., JCI, 2018). Additionally, stereotactic body radiation therapy (SBRT) is being investigated as a tool for improved immune recognition and preconditioning for immune checkpoint blockade in RCC. As such, we sought to identify HERVs expressed subsequent to SBRT in RCC. A clinical trial at Roswell Park Comprehensive Cancer Center was performed in which patients diagnosed with clear cell RCC were treated with 15 Gy SBRT to the primary tumor 4 weeks prior to nephrectomy (NCT01892930). Tumor samples from patients enrolled on this trial as well as control RCC samples from patients treated with nephrectomy only were preserved in single cell suspensions. Prior analysis from our group of these samples showed an increase in tumor-associated antigen expression following SBRT. We analyzed single cell RNA sequencing data from these same samples to evaluate HERV expression. CD45 negative cells were isolated computationally and collapsed for bulk transcriptomic analysis. Utilizing differential expression analysis of known HERV transcripts, several HERVs were found to be significantly upregulated in patients treated with SBRT compared to nephrectomy only, α (alpha) = 0.05: ERV3-1, ERVMER61-1, and ERVW-1. Current characterization of HERV transcripts within publicly available reference databases is limited, therefore we next will apply a validated computational algorithm, hervQuant, to examine a more robust directory of HERV sequences in the context of SBRT in RCC. Through this work, novel immunogenic targets upregulated in tumor cells by existing treatment approaches can be identified for further investigation.

Poster No. 39

Treponema denticola Lipoprotein Dentilisin is Crucial for The Development of Aortic Endothelium Dysfunction

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Objective: Periodontitis (PD) affects approximately 47% of the population in the United States and is a potential risk of various systemic diseases, including cardiovascular diseases (CVD) involving inflammatory endothelial cell (EC) dysfunction. PD is associated with dental plaque biofilm community changes, including prevalence of *Treponema* spp such as T. *denticola* (T.d) which has also been detected in atherosclerotic plaques and vasculature during CVD. Bacteria may promote vascular inflammation via direct interaction with ECs or indirectly through systemic involvement of bacterial products and inflammatory mediators. T.d-exposure promotes secretion of the pro-inflammatory cytokine Oncostatin M (OSM) from neutrophils. OSM and neutrophils are both associated with PD and have been identified as potential contributors to CVDs. Neutrophils are primary producers of OSM, which is elevated in saliva and serum during PD and in serum during CVD. Despite the role of T.d in PD development and detection in atherosclerosis lesions, the interaction with ECs and contribution of specific virulence factors to promote a pro-inflammatory vascular environment remains understudied. This research aims to study how *Treponema* spp exposure modulates OSM production and define how the *T. denticola* protease dentilisin mediates pro-inflammatory endothelial cell changes.

Methods: Human aortic endothelial (HAoEC) cells were treated with Td (MOI 50) for 24hrs. Human or mouse neutrophils were treated with *Treponema* species or a *T. denticola* dentilisin mutant for 3hrs. RNA was isolated to measure gene transcription by qPCR, and cellular lysates and supernatants used to assess cellular signaling by Western blot or cytokine secretion by ELISA. Dextran permeability of ECs was assessed following Td exposure. Neutrophil transmigration across bacterial pre-exposed (6hrs) HAoEC was assessed using immunofluorescence.

Results: T.d promotes endothelial adhesion factor expression, and changes in the adherens junction component VE-cadherin. T.d lipoprotein protease dentilisin promotes increased IL-8 secretion, vascular permeability, neutrophil transmigration together with decreased expression of the tight junction component Occludin. Diverse *Treponema* spp. promote OSM secretion from neutrophils.

Conclusion: *T. denticola* lipoprotein dentilisin is crucial for the progression of a vascular inflammatory environment in vitro via activation and permeability of EC and dysregulation of adherens and tight junction complexes. This may contribute to the risk and development of CVDs in atheroprone environments.

Poster No. 40

High dimensional flow cytometry reveals radiation-induced cellular changes in human renal cell carcinoma

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Spectral flow cytometry is a powerful high dimensional mass cytometry technique that can be used to study more than 40 markers simultaneously. While conventional cytometers rely on single channel emission detection systems, spectral flow cytometers read the full spectrum to better distinguish fluorophores that have overlapping emission channels thereby allowing them to be used in the same panel. Here, we developed a 35-marker lymphocyte panel and performed complementary single cell RNA sequencing to evaluate the tumor-immune landscape of renal cell carcinoma patient tumors that had been treated *in situ* with high-dose radiation. Our analysis revealed an increased transition from naïve/stem-like $CD8^+$ T cell populations to more exhausted/effector phenotypes in irradiated tumors. Single cell RNA sequencing confirmed these findings and further revealed an increased expression of CD8 ligands, TRAIL and INFγ, in radiated samples. Analysis of publicly available transcriptional data from patients treated with immunotherapy revealed those with high TRAIL and IFNγ expression had longer overall survival. These observations provide an indication of how radiation remodels the human tumor immune landscape and potentially influences the interaction between $CD8^+$ T cells and cancer cells. We are currently optimizing a 44-marker spectral flow panel to assess the changes in the myeloid compartment. Put together, our lymphoid and myeloid panel will serve as powerful tools to study immune responses in patients receiving different treatment modalities.

Poster No. 41

Sexual Dimorphism Drives ILC2 Function in Lung Adenocarcinoma

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The prevalence of non-small lung cancer (NSCLC) has historically been greater among males in the US, however, the pattern of incidence between males and females has now shifted. Over the last few decades, incidence rates of lung adenocarcinoma (LUAD) continue to rise and disproportionately affect the female population. Recent epidemiological studies, along with our analysis from the US National Cancer Database, found female incidences of NSCLC had surpassed males by 5-15% in groups under the age of 50, and cannot be explained by differences in smoking behaviors. To characterize LUAD in males and females, we generated a spontaneous LUAD murine model using double mutant $Kras^{G12D}$;$Trp53^{fl/fl}$;adenovirus-cre-GFP. When we compared tumors generated from our model, we found an increase in both type 2 immune inflammation and tumor proliferation in the female tumors. Many studies on sex-biases in autoimmune and inflammatory diseases highly suggest the regulation of sex hormones in the immune system, however, the molecular mechanisms behind the sexual dimorphic immune response in LUAD remains unknown. One of the first responders of the type 2 inflammation are type 2 innate lymphoid cells (ILC2), that are recognized as orchestrators of various downstream immune responses. In our transcriptomic analysis of non-tumor bearing mouse lung ILC2s, we observed differential gene regulation between sexes with the upregulation of ILC2 signatures in female ILC2. This study will be one of the first to investigate the role of sexual dimorphism in ILC2 function in the context of cancer. Collectively, the identification of the mechanism in ILC2 activation has potential long-term therapeutic implications in personalized medicine, and further present a new paradigm about the role of biological sex and lung tumorigenesis.

Poster No. 42

Characterization of the lung B cell antibody response in people with cystic fibrosis and respiratory nontuberculous mycobacteria infection

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Nontuberculous mycobacteria (NTM) are an increasingly common cause of respiratory infection in people with cystic fibrosis (PwCF). Relative to those with no history of NTM infection (CF-NTM^{NEG}), PwCF and a history of NTM infection (CF-NTM^{POS}) are more likely to develop severe lung disease and experience complications over the course of treatment. We recently reported lung lobe-specific immune profiles of 3 cohorts (CF-NTM^{POS}, CF-NTM^{NEG}, and non-CF adults) and found that the CF-NTM^{POS} airways are distinguished by accumulations of B cells and the cytokines that support their survival and division (sCD40L, IL10, IL5, BCA-1). These cells and cytokines were enriched in the most damaged lung lobes. Here, we describe the approach we are taking to identify which antibody isotopes these lung B cells produced and whether any react against self-antigens (i.e., autoantibodies), as well as the data we have generated to date to test this possibility. The presence of autoantibodies would provide a mechanistic connection between B cell abundance and lung tissue damage. We summarize the literature regarding autoantibodies in PwCF, which although limited supports the possibility that autoantibodies are found more frequently in the sera of PwCF, and provide a conceptual model for how the presence of NTM and excess antibody concentrations may relate to one another. We anticipate these findings will inform the development of host-directed therapies to improve NTM disease treatment in PwCF.

Poster No. 43

Analysis of lipid uptake and lipid droplets in primary murine Group 2 Innate Lymphoid Cells

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Group 2 Innate Lymphoid Cells (ILC2s) are critical drivers of innate and adaptive type 2 immune responses, conferring resistance to helminth infections and orchestrating tissue restorative and wound healing processes. In addition, ILC2 have been shown to be implicated in chronic inflammatory barrier disorders including type 2 immunopathologies such as allergic rhinitis and asthma. ILC2s influence local as well as systemic metabolism. In turn, the metabolic status shapes ILC2 phenotypes and effector functions. Determining the metabolic requirements of ILC2s is critical for our understanding of ILC2's roles in type 2 immune responses and its associated pathophysiologies. ILC2s have recently been shown to be rich in lipid-storing organelles called lipid droplets (LDs). However, many mechanisms of LD turnover, lipid metabolism and catabolism and its implications regulating ILC2 phenotypes and effector functions remain largely unknown.

Utilizing flow cytometry and confocal microscopy we detail here novel and complementary experimental approaches to study fatty acid uptake and LD turnover in primary murine ILC2s and link them to phenotypes and functionality. In addition, we complement these assays with novel assays in confocal microscopy to investigate organelle morphology and localization of mitochondria, lysosome and LDs in ILC2s. Linking lipid metabolism networks and LD turnover to ILC2 phenotypes and effector functions is critical for the assessment of novel pharmaceutical strategies to counteract ILC2 functions in type 2 immunopathologies.

Poster No. 44

Characterizing Innate-like CD8$^+$ T Cells in Early Life

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The immune system is classically divided into two categories: innate and adaptive, where the former responds rapidly and nonspecifically and the latter does so more slowly, precisely, and with greater speed and specificity over time. These conceptual distinctions are most appropriate for adult humans, who have a substantial repertoire of mature naïve lymphocytes that can establish immunological memory to protect against reinfection. Because the infant immune system continues to develop after birth and does not yet have an archive of memory cells, it must be capable of rapid and often nonspecific responses to signals of danger and damage. T cells are crucial to proper functioning of the adaptive immune system and are responsible for recruiting and coordinating immune cells, shaping future responses, and directly killing infected or cancerous cells. CD8+ T cells are canonically known for their cytolytic functions but can also produce cytokines under certain inflammatory conditions. During the last two decades an array of lymphoid immune cells have been described as having innate-type functions and/or a strongly restricted capacity for recognizing foreign antigens. More recently, we have found evidence for a subset of CD8+ T cells that arise *in utero* during the second trimester, persist variably into adulthood, and behave in an innate-like manner. We report the initial flow cytometric characterization of the phenotype and function of putative fetal innate-like CD8+ T cells (FITs) derived from cord blood mononuclear cell samples.

Poster No. 45

Treating Metastatic Pancreatic Ductal Adenocarcinoma with SBRT and IL-12 mRNA

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Pancreatic ductal adenocarcinoma (PDAC) carries a median survival of less than 1 year. This poor outcome primarily stems from the late presentation of this disease, with more than 50% of patients diagnosed in the metastatic setting. Metastatic PDAC has no viable treatment options and the use of different chemotherapy and radiation regimens in this setting leans more to quality-of-life advantages than benefits in survival. Hence, treatments are greatly needed in this disease subset.

Stereotactic body radiation therapy (SBRT) utilizes higher doses (20- 35 Gy) over smaller fractions (5- 6 fractions) than conventional radiation and has largely replaced its predecessor due to demonstrated benefits in safety, down-staging capabilities and survival in the clinic. Moreover, we have recently shown that SBRT is a strong inducer of immunogenic cell death and could elicit T cell clonal expansion in a subset of PDAC patients, suggesting it has the potential to *initiate*- but not *maintain*- anti-tumor immune responses.

While the benefits of SBRT alone are modest in PDAC, combining this treatment with immunotherapies is an attractive proposition. One such treatment being tested by our group is the pleiotropic cytokine IL-12 in the form of a mRNA lipid nanoparticle encoding both subunits of the heterodimer. IL-12 can act on a multitude of immune cells in the tumor microenvironment, potentially fostering anti-tumor immune responses by eliciting CD8+ and CD4+ T cell effector function while also repolarizing immunosuppressive myeloid cells to a more immune stimulatory phenotype. Hence, we hypothesized that combining SBRT and IL-12 mRNA in PDAC would both *initiate* and *maintain* anti-tumor immune responses.

When we deliver SBRT/IL-12 therapy locally to an orthotopic, murine PDAC tumor, we see durable responses leading to cures in this model. Additionally, these cured mice could reject a subsequent rechallenge one month later, leading us to speculate that we could also treat metastatic disease. For this, we developed a murine model of metastatic PDAC that recapitulates human disease by injecting PDAC cells through the portal vein to colonize the liver concurrently when inoculating the pancreas tumor. Interestingly, although SBRT/IL-12 was administered to the primary tumor only, we noted significant anti-tumor effects to liver metastases that translated to cures in the majority of these mice. Additionally, we also explored an innovative means of treating liver metastasis directly with SBRT/IL-12 and could show downsizing effects on primary disease, potentially opening a new avenue for metastatic PDAC treatment.

Poster No. 46

Characterization and genome sequence of *Mycobacterium intracellulare* ABSURDO, a novel pathogenic isolate with three colony morphotypes that vary in growth and acid-fastness

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Mycobacterium intracellulare in a nontuberculous mycobacteria (NTM) species which can cause serious and sometimes fatal disease in immunocompromised individuals. Other NTM species, including *M. avium* and *M. abscessus*, commonly exhibit two colony morphotypes (smooth and rough) which vary in appearance and liquid growth properties. Here we characterize a novel clinical isolate of *M. intracellulare* which exhibits three (not two) colony morphotypes which differ not only in appearance and liquid growth properties, but also their acid-fastness and *in vivo* survival following infection of mice via an inhalational exposure model. The genome of this isolate, which we have termed ABSURDO, as well as the genome of each morphotype components, aligns with that of *M. intracellulare* but yet contains ~16% more protein coding sequences (5980-5999 depending on the ABSURDO morphotype) than the *M. intracellulare* type strain ATCC 13590^{T}. Polymorphism analysis of each morphotype genome revealed that across the three morphotypes there were only two mutations which had a high likelihood of causing a phenotype due to a genetic change: one in the gene encoding the two-component system response regulator MtrA, and another in that which encodes modular polyketide synthase. Neither of these genes have been previously implicated in the morphotype shifting of *M. avium* nor *M. abscessus*. In summary, *M. intracellulare* ABSURDO is a novel pathogenic isolate which fulfills Koch's postulates, the genome of which aligns with but is nevertheless unique from a *M. intracellulare* type strain and has three morphotype components which differ in two genes that have never been implicated in colony appearance, growth or acid-fastness.

Poster No. 47

Gut IgA+ Plasma Cells Differentially Utilize Metabolism to Regulate Antibody Secretion

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IgA secreting plasma cells (PCs) in the gut provide durable humoral immunity by supplying critical antibodies for barrier function against pathogenic and commensal bacteria. PCs are historically understudied and have a very specialized function. IgA+ PCs can be found in the bone marrow (BM) and gut niches and have unique tissue-driven transcriptional profiles. However, in the gut niche, the cell-intrinsic and -extrinsic signals responsible for regulating function remain largely unknown. We found that IgA+ PCs in the gut secrete significantly fewer antibodies on a per-cell basis compared to bone marrow. Recent studies have established that the metabolic program of a PC determines antibody secretion in the bone marrow niche. To assess if this difference in functional capacity could be due to metabolic consequences, we evaluated the metabolic profile of the tissue-resident IgA+ PCs. Interestingly, gut IgA+ PCs have an increased glycolytic capacity, while BM IgA+ PCs have increased mitochondrial dependence. IgA+ PCs have significantly more mTOR activity and low levels of cellular ROS in the gut niche, supporting a glycolytic phenotype. Further, we found antibody secretion in the gut is selectively sensitive to glycolytic inhibition. We hypothesized that the proximity of IgA+ PCs to nutrients in the gut could be contributing to their observed phenotype. Recent studies have shown that short-chain fatty acids (SCFAs) regulate cellular glycolytic activity. Therefore, we introduced a fiber-free diet, thereby limiting exposure to SCFAs, and we assessed if this would lead to functional PC changes. When fiber was removed from the diet, antibody secretion selectively decreased in gut IgA+ PCs. Herein, we observed that differential metabolism regulates PC function in the gut. We aim to further dissect the role of specific nutrients in driving gut IgA+ PC metabolism and function. Ultimately, understanding how antibody secretion is regulated in the gut provides a significant tool for sustaining intestinal homeostasis, targeting mucosal responses against pathogens, and will advance strategies in developing mucosal vaccines.

Poster No. 48

Induction of Systemic IgA Responses by the Human Commensal *Bacteroides fragilis*

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IgA antibodies are the most abundantly produced antibody isotype and are excreted at mucus membranes to maintain barrier function. The generation of IgA secreting plasma cells in mucosal tissues has been thoroughly explored, however, the factors that dictate the generation of systemic IgA responses outside this compartment have remained enigmatic. Recent studies have established a role for commensal bacteria in shaping systemic IgA responses, however, the specific species involved and the regulatory mechanisms of this induction are not well-understood. *Bacteroides fragilis*, a Gram-negative anaerobe ubiquitously found in the intestinal tract of humans, is a robust inducer of secretory IgA and a primary mediator of intestinal homeostasis through modulation of Th17-Treg balance. We found that mice treated with *B. fragilis* via oral gavage generate bone marrow-resident IgA plasma cells and serum IgA specific to this organism in a dose-dependent manner. Using *B. fragilis* as a model for exploring the mechanisms of systemic IgA induction, we have shown that this process is dependent on T cell-B cell interactions and germinal centers. Additionally, we determined that small intestinal Peyer's patches are required for the generation of *B. fragilis*-specific bone marrow IgA plasma cells, which suggests this lymphoid structure may function as a vital bridge between symbiotic gut microbes and systemic immune responses. Furthermore, our data indicates that systemic IgA induction is intimately linked with the nature of *B. fragilis* colonization and subsequent exposure to the epithelial layer and immune tissues of the small intestine. This data indicates that generation of systemic IgA is inextricably linked with the species composition of the intestinal microbiota and the colonization dynamics of this complex ecosystem. Lastly, our data shows that serum IgA specific to *B. fragilis* is protective against the formation of polymicrobial peritoneal abscesses, thus providing additional clinical relevance to organism-specific systemic IgA responses. Collectively, this work provides the first evidence of a mechanism of systemic IgA induction by a single species and will help us further our understanding of how gut microbes dictate immunological function beyond the gut compartment.

Poster No. 49

Differentiating Cell Intrinsic and Extrinsic Features of Antibody Dependent Enhancement in DENV Infection

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Dengue virus (DENV) is a flavivirus consisting of four distinct serotypes (DENV1-4) co-circulating in endemic areas. Upon secondary infection with a heterologous serotype, cross reactive sub-neutralizing and non-neutralizing antibodies formed in response to the primary infection may facilitate viral entry via phagocytosis, a process termed antibody-dependent enhancement (ADE). ADE contributes to increased virion and pro-inflammatory cytokine production and has been associated with increased disease severity. However, it is unclear as to whether these effects are purely a result of increased viral load, or whether this alternative route of viral entry alters host cell transcriptomic responses to facilitate viral replication. It has been reported that compared to canonical receptor-mediated entry, entry via ADE induces a qualitatively different response by the host cell, coined "intrinsic ADE". However, evidence supporting intrinsic ADE have been controversial, and largely limited by the ability to specifically characterize infected cells. Our group has previously demonstrated the successful capture of DENV transcripts in infected cells using 5' single cell RNA sequencing (scRNA seq). In this work, we aim to develop a similar method to identify infected cells and subsequently explore transcriptomic differences according to route of viral entry. We have successfully developed a methodology using 3' capture scRNA seq to identify and transcriptionally characterize infected cells. Next, we will apply this technique to characterize transcriptomic profiles of monocyte-derived macrophages infected by typical receptor mediated entry compared to those infected by ADE. This work will contribute to our understanding of ADE as a critical mechanism driving disease severity, providing insight into potential opportunities for intervention.

Poster No. 50

Complement signaling as a T-cell checkpoint in the ovarian cancer tumor microenvironment

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Multiple immunosuppressive pathways are obstacles to T cell-driven antitumor immunity. In ovarian cancer, this includes the distinct populations of suppressive immune cells in the fluid ascites microenvironment. Patients with advanced stage ovarian cancer normally present with increased accumulation of ascites fluid, a consequence of leaky blood vessels. The ascites however is an injurious and inflammatory microenvironment, rich in secreted components from tumor cells, DAMPS, cytokines and complement activation products. Using OC ascites supernatants as an authentic component of human TME, we have identified that normal, circulating neutrophils acquire a suppressive phenotype measured by suppression of CD3/CD28 stimulated T cell proliferation. T cell suppression is dependent on neutrophil-T cell contact and a number of neutrophil functions that include complement signaling and NADPH oxidase. Neutrophil suppressors also have broad effects on pathways driving T cell activation including inhibition of cytokine responses, metabolic changes and mTOR activation. Separate from effects of suppressor neutrophils are the effects of the immunosuppressive ascites microenvironment on T cell function. Exposure of CD3/CD28 stimulated T cells to ascites dramatically reduces T cell cytokine responses (IL-2, IFN-y) however, has no effect on T cell proliferation. Complement activation drives neutrophil suppressors but its role in T cell activation and fitness in the ascites microenvironment is not well studied. We therefore asked if complement signaling would have on T cells in the TME, independent of neutrophils. Exposure of stimulated T cells to ascites augmented C3 mRNA levels. As T cells themselves can be a source of complement in the TME, we employed multiple small molecule inhibitors targeting complement activation and signaling via receptors at different levels to identify a role for complement in T cell cytokine responses. Stimulated T cell cytokine production was rescued by inhibiting complement activation, specifically the alternative pathway. However, signaling via complement receptors C3aR and C5aR do not play a role in modulating T cell cytokine responses. Our results point to a complement activation-dependent suppression of T cell responses in the TME driven by alternative pathway activation. Future studies will be aimed at zeroing in on specific pathways (e.g., CD46-mediated signaling, downstream NFAT/ NFKB signaling) to determine how complement alters T cell responses in the TME.

Poster No. 51

Prenatal exposures to per- and polyfluoroalkyl substances (PFAS) and its impact on the developing neonatal immune system

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Per- and polyfluoroalkyl substances (PFAS) are persistent synthetic chemicals widely used in industrial processes and consumer goods. Their highly stable nature has made them abundant and persistent in the environment and detectable in every living organism. PFAS present in the maternal circulation during pregnancy are known to cross the placenta and accumulate in the fetus, as evidenced by measurable PFAS in cord blood and a steep reduction in post-pregnancy maternal PFAS levels. There is compelling evidence of PFAS-associated immunotoxicity vis-a-vis lowered antibody responses to vaccination; however, little attention has been given to how these toxicants might affect the development of infant cellular immunity, particularly T cells. Fetuses and infants are particularly sensitive to environmental exposures due to their rapid growth and developing systems. During fetal development, each immune cell type develops at different gestational stages, thus perturbations during pregnancy or in the early postnatal period could affect multiple types of immune cells. To test the hypothesis that maternal PFAS disrupts infant T cell development, a cohort of 286 pregnant women in Rochester NY, were enrolled between 2015 and 2019. Second-trimester maternal PFAS concentrations were determined by high-performance liquid chromatography (HPLC) and tandem mass spectrometry (MS/MS). Infant immune population frequencies were measured at birth, 6, and 12 months of age using mass cytometry (n= 284 samples, 199 subjects). Results show the phenotypic and time-dependent complexity within the $CD4^+$ and $CD8^+$ T cell pool and the presence of 36 unique $CD4^+$ and 14 unique $CD8^+$ clusters of cells identified through high-dimensional analysis. We further show that higher PFAS concentrations are associated with lower IL-21 producing $CD4^+$ T cells. IL-21 is a cytokine essential for the germinal center reaction and critical for T cell – B cell interactions to support antibody affinity and maturation. Thus, our data suggest a possible link to suppressed antibody responses associated with PFAS exposure. Next steps include incorporating known confounders to our model and further phenotyping of the IL-21^+ $CD4^+$ T cell populations with a targeted mass cytometry panel, including T follicular helper cell markers. These findings provide new insight into the complexity of the immune response during infancy, and the impact of antenatal exposures on reshaping the fetal developmental program.

Poster No. 52

Assessing the effect of *Bifidobacterium* on *in vitro* Immune Responses

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Background: Atopic diseases have been one of the leading causes of chronic illness over the past 50 years. The "Hygiene Hypothesis" purports that this is due to the shift from farming to an industrial lifestyle, reducing exposure to key microbes. Studies in our lab show that members of the Older Order Mennonites (OOM) have reduced allergy rates and altered microbiome composition. Of interest is *Bifidobacterium infantis* (*B. infantis*), a gut commensal organism that makes up a significant portion of the microbiome of breastfeeding OOM infants. We are interested in investigating the potential effects of novel *B. infantis* strains we have isolated and non-*infantis Bifidobacterium* species on modulating immune responses.

Methods: We collected supernatants from *B. infantis* and other infant-associated *Bifidobacterium (B. bifidum, B. longum, B. breve)* strains grown using human milk oligosaccharides as the sole carbon source to test if secreted metabolites impact cytokine levels produced in the gut epithelium and immune cells alone and in response to TLR agonists. We treated polarized monolayers of Caco2 epithelial cells, grown in transwells, for 4 hours with either TLR agonists (LPS, Flagellin, or PAM3CSK4), supernatants, or a combination and measured cytokine (IL-8) expression levels using qRT-PCR. We also treated monocyte-derived dendritic cells (MDDCs) for 48 hours with the same conditions and measured levels of 13 cytokines using a multiplex assay.

Results: *B. infantis or* non-infantis *Bifidobacterium* supernatant did not impact Caco2 cells baseline IL-8 expression levels and was not able to significantly reduce the IL-8 expression levels induced by LPS, PAM3CSK4, or Flagellin. The MDDC assays showed that some of the *B. infantis* and *Bifidobacterium* supernatants were able to significantly reduce levels of agonist-induced inflammatory (IL-2, IL-6, IL-8, MCP-1, and TNF-α) and regulatory (IL-10) cytokines ($p<0.05$-0.001), while *Bifidobacterium* also resulted in significantly increased levels of IFN-y, IP-10, IL-17, and IL-12 at baseline ($p<0.01$- 0.001).

Conclusions: *B. infantis* and *Bifidobacterium* supernatants significantly reduced inflammatory cytokine production induced by TLR agonists in immune cells but not epithelial cells. Non-infantis *Bifidobacterium* increased levels of Th1 T-Cell polarizing cytokines produced from MDDCs, an effect not seen with *B. infantis.* These data suggest that many *Bifidobacterium* may elicit immunomodulatory effects, which result in reduced inflammatory cytokine, but non-infantis *Bifidobacterium* increased Th1 cytokine production, associated with dampening an allergic response.

Poster No. 53

Assessing the Relationship Between Maternal Milk and Infant Fecal IgA Levels and Allergic Development in Urban vs Rural Environments

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Background: Previous research suggests that increased levels of immunoglobulin A (IgA) offer protection against food allergy. IgA is the predominant immunoglobulin present in maternal milk, with breastfeeding thought to be the initial mechanism for infant IgA production. Traditional farming communities, like the Old Order Mennonites (OOM) of Upstate NY, show lower prevalence of allergies; however the mechanism is not known. This study examines the IgA levels in urban (ROC) vs rural (OOM) mothers' milk and their infants' stool samples.

Methods: The samples were obtained from subjects in our birth cohort study, conducted in mothers and infants from the Rochester and Mennonite communities. Maternal milk samples were collected at 6 weeks (*N=130)* and 6 months *(N=125)*. We collected fecal samples from paired ROC and OOM infants at 6 weeks (*N=118)*, 6 months *(N=138)* and 12 months *(N=130)*. ELISA assays were used to measure the total IgA1 and IgA2 levels. LUMINEX assays were used to measure the specific IgA1 and IgA2 levels in human milk for 6 different food antigens.

Results: Human milk IgA levels decreased over time, but OOM mothers had higher total IgA1 levels than ROC mothers at 6 weeks (p=.03), whereas ROC mothers had significantly higher IgA levels for casein at 6 weeks (p=0.0003) and 6 months (p=0.006) and to peanut at 6 months (p=0.015). OOM infants had higher fecal IgA1 levels at 6 weeks (p=1.5e-09), 6 months (p=.056) and 12 months (p=.004) and higher IgA2 levels at 6 weeks (p=.0001), and 6 months (p=2.4e-05).

Conclusions: The results obtained from this study further demonstrate the relationship between mucosal IgA levels and different lifestyles. We can conclude that a farming lifestyle has a positive effect on IgA levels in human milk and infant stools, but their association with development of allergic diseases remains to be further explored.

Poster No. 54

Diversity of the γδ TCR Repertoire Diminishes with Age and Cancer

Authors: Pranav Murthy[1], Daniel Weber[2], Sagar Sharma[1], Jennifer Bone[3], Wenjing Pan[2], Renee Anderko[1], Amer Zureikat[1], Miranda Byrne-Steele[2], Jian Han[2], Michael T. Lotze[1,2]

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The diversity and clonal hierarchy of the stochastically recombined adaptive immune repertoire are critical determinants of the development and outcome of human disease. While the repertoire of αβ T cell receptor (TCR) expressing cells has been well characterized, the MHC independent γδ TCR repertoire has yet to be fully elucidated. Utilizing seven chain (adaptome) dimer avoidance multiplex-PCR and next generation sequencing, we investigated the landscape of the circulating γδ TCR repertoire in 237 healthy individuals with no known underlying diseases or evidence of active infection. 1,176,406 total Vγ and 634,751 Vδ CDR3 sequences were analyzed, identifying 216,147 Vγ and 182,840 Vδ unique CDR3s (uCDR3s). γδ TCR sequences accounted for an average of 4.2±3.4% of the total TCR and BCR repertoire, ranging from 0.37% to 19.9%. The Vδ chain, containing diversity (D) gene segments, was more diverse by multiple diversity indices ($p<0.0001$) compared to the paired Vγ chain. The Vδ2 repertoire was dominant, but certain individuals showed a high frequency (>50%) of Vδ1 and Vδ3 CDR3s. Using the inference and generation of repertoire (IGoR v1.4.0) algorithm, we trained unproductive Vδ and Vγ CDR3 sequences to develop statistical models of V(D)J recombination that were used to calculate the generational probability of productive CDR3 sequences, with higher probabilities associated with more random and potentially public CDR3s. The frequency of Vδ1 sequences was negatively correlated ($r=-0.77$, $p<0.0001$) with Vδ1 generational probability and diversity, indicative of donor-specific adaptive clonal expansions. There was no association between sex, BMI, tobacco, or alcohol history and γδ expression or diversity. Despite stable overall expression, an age associated reduction in γδ TCR diversity ($r=-0.34$, $p<0.0001$) was observed that was more apparent than corresponding αβ ($r=-0.11$, $p=0.086$) or B cell ($r=-0.11$, $p=0.065$) repertoire diversity change with age. This reduction in γδ peripheral diversity could be attributed to loss of uCDR3s and increases in the frequency of the most dominant clonotype with age. The diversity of both the Vγ and Vδ chains was also dramatically reduced in the peripheral blood of age matched patients with solid tumors ($p<0.0001$), including those with kidney (n=29), and head and neck cancer (n=28). Collectively, these results support age and cancer associated perturbations of γδ T cell diversity and immunosurveillance that should be considered in the development of novel immunotherapies.

Poster No. 55

CAR T cells armored with Super IL2 and IL33 reshape the tumor microenvironment to control solid tumor growth

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Chimeric antigen receptor (CAR) T cell efficacy against solid tumors is challenged by key obstacles within the tumor microenvironment (TME). These include tumor-intrinsic expression of inhibitory ligands that contribute to immune evasion, the absence of essential nutrients required for T cell survival, and the presence of tumor associated immunosuppressive cells. To bolster CAR T cell resistance to immunosuppression and enhance anti-tumor activities of endogenous immune cell populations, CAR T cells were armored with an IL-2 superkine (Super2) and alarmin IL-33. Super2 and IL33 expressing CAR T cells were adoptively transferred into immunocompetent mice with established primary or metastatic B16F10 melanoma, intradermal MC38 colon carcinoma, or intraperitoneal ID8 ovarian cancer. We show that a single dose of Super2-IL-33 CAR T cells promoted the control of both metastatic and solid tumors in mice without lymphodepletion or preconditioning regimens.

The expression of a tumor specific CAR or cytotoxic effector molecules by adoptively transferred T cells expressing Super2 and IL33 were dispensable for tumor control, underscoring the contribution of endogenous immune cells in mediating therapeutic efficacy. To better understand the effects Super2 and IL33 armored CAR T cells on endogenous immune cell populations, we analyzed tumor infiltrating lymphocytes (TILs) in treated and non-treated tumors. Expression of Super2 and IL33 led to the expansion of both CAR T cells and endogenous lymphocytes. Armored CAR T cell treatment led to increased frequencies of M1 macrophages and effector CD8 T cells in tumors compared to non-armored CAR T cell treated and non-treated tumors. This shift resulted in an increased ratio of CD8 effector T cells to regulatory T cells. Additionally, expression profile analysis of tumor infiltrating regulatory T cells from non-treated and Sup2 and IL33 CAR T cell treated tumors revealed several differentially expressed genes that have been linked to decreased regulatory T cell activity. Future studies aim to determine if these changes in gene expression result in functional changes in regulatory T cells that ultimately contribute to the tumor control mediated by Super2 and IL33 armored CAR T cells. Together, these findings show that CAR T cells armored with Super2 and IL33 broadly alter endogenous immune cell populations resulting in control of solid tumor growth.

Poster No. 56

Evaluating the efficacy of equine mesenchymal stromal cell secretome on innate immune responses *in vitro* and *in vivo*

Authors: Aarthi Rajesh[1], Kelly Oxford[1], Rebecca Harman[1], and Gerlinde Van de Walle[1]
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Chronic wound infections continue to be a significant burden in equine medicine and treatment has become particularly challenging due to the increasing prevalence of antibiotic-resistant bacteria. The presence of antibiotic-resistant bacteria, such as Methicillin-resistant Staphylococcus aureus (MRSA), may delay healing by impairing immune cell functions. This study aimed to investigate the potential of the equine mesenchymal stromal cell (MSC) secretome to improve equine innate immune responses.

First, a series of *in vitro* assays were done to evaluate the impact of the MSC secretome, collected as conditioned medium (CM), on equine neutrophil and macrophage functions. There was a significantly increased chemotaxis, but decreased phagocytic ability, of neutrophils when treated with MSC CM compared to controls. MSC CM treatment increased polarization of macrophages to both M1 and M2 phenotypes. Neutrophil and macrophage ROS production showed an increased trend, albeit not statistically significant, when treated with MSC CM. The efficacy of the MSC secretome on wound healing, with an emphasis on innate immune cell infiltration, was next evaluated *in vivo* using a full-thickness mouse wounding model. Wounds treated with MSC secretome showed accelerated wound closure compared to controls, characterized by enhanced granulation tissue formation and resolution, increased vasculature, regeneration of hair follicles, and importantly, increased neutrophil and macrophage infiltration. Collectively, our findings indicate that the MSC secretome impacts innate immune cell functions *in vitro* and *in vivo* , suggesting that the equine MSC secretome might have the potential to restore impaired immune cell functions in infected wounds.

Symposium IV

Clinical Immunology

Chair: Michael Robek

"Structures of human antibodies bound to multiple neutralizing epitopes on HCV glycoprotein E2 inform vaccine design efforts"

Marty Schoenle[1], Xander Wilcox[1], Clinton O. Ogega[2,3], Nicole E. Skinner[4,5], Nicole Frumento[2], Desiree A. Wright[2], Harry T. Paul[2], Ariadne Sinnis-Bourozikas[2], Kaitlyn E. Clark[2], Alexis Figueroa[2], Pamela J. Bjorkman[6], Stuart C. Ray[2,3], Justin R. Bailey[2], Andrew I. Flyak[1]

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Background: From the structural studies of E1E2 glycoprotein-specific broadly neutralizing antibodies (bNAbs) isolated from HCV-infected individuals, we learned that the front layer of E2 glycoprotein (antigenic region 3, AR3) is the main target of *VH1-69*-encoded bNAbs. Two regions on E2 are thought to contain either non-neutralizing epitopes (AR1 in the E2 b-sandwich) or epitopes targeted by antibodies with a narrow neutralizing breadth (AR2 in E2 back layer). While bNAbs that bind E1-stabilized metastable epitopes in the back and stem region of E2 (AR4A-like bNAbs) are elicited in individuals who clear HCV, E1E2 heterodimer expression and purification for HCV vaccine studies remain a big challenge due to protein oligomerization and aggregation. The information about the existence of potent bNAbs targeting non-AR3 neutralizing epitopes on E2 can inform the design of easier-to-produce E2 immunogens that could elicit synergistic antibody responses.

Methods: We used longitudinally-collected blood samples from an HCV Elite Neutralizer who cleared multiple infections to isolate bNAbs recognizing non-overlapping E2 antigenic regions. We characterized the gene usage of the isolated bNAbs and determined their neutralization potency and breadth. To determine the epitope specificity of the bNAbs, we performed epitope binning experiments using Octet BLI biosensors and binding experiments with E2 knockout probes. Finally, we determined crystal structures of E2 glycoproteins complexed with selected bNAb Fabs (hcab40, hcab55, hcab64, and hcab17) representing key E2 antigenic regions.

Results: Epitope mapping of E2-specific bNAbs revealed three major antigenic regions, with potent bNAbs being represented in each region. The structural analyses of E2 complexed with bNAb Fabs from each antigenic region revealed that isolated bNAbs use diverse V_H-genes to target AR3 (*VH1-46* encoded hcab55 and hcab64), AR2 (*VH4-34* encoded hcab40) and AR1 (*VH4-34* encoded hcab17). The structures of the front layer-specific *V_H1-46* bNAbs, hcab55, and hcab64, were remarkably similar to the previously described crystal structure of AR3-specific *V_H1-69* bNAb HEPC74, which was also isolated from the same subject. AR1-specific hcab17 and AR2-specific hcab40 competed for binding with several bNAbs, including the ultrapotent bNAb hcab5, indicating that both AR1 and AR2 contain neutralizing epitopes. Finally, the structural analysis of the Hcab40-E2 complex indicated that the hcab40 binding footprint spanned both AR2 and recently characterized AR4A epitope in the stem region of E2. However, unlike E1E2-specific AR4A, hcab40 did not require E1 stabilization of E2 for binding, suggesting that AR2/AR4 interface might represent a novel target for E2-based immunogen design.

Conclusions: The existence of antibodies that target non-overlapping neutralizing E2 epitopes in humans suggests the possibility of a synergistic antibody response between bNAbs that utilize multiple V_H-genes. The structural characterization of novel AR1 and AR2 bNAbs will facilitate lineage-targeted E2-based immunogen design to induce the development of bNAbs to multiple conserved epitopes.

"Immunology in a Public Health Setting"

William Lee, Ph.D.

Wadsworth Center/New York State Department of Health

This presentation will be an overview of the Wadsworth Center and, in particular, the Diagnostic Immunology Laboratory (DIL) at the Wadsworth Center. Although the Wadsworth Center scientists have participated in NYIC since its inception, the focus has generally been on the extensive basic research and affiliation of the Wadsworth scientists with the University at Albany School of Public Health. This talk will instead describe the role that immunology plays in contributing to public health diagnostics and, especially, infectious disease outbreaks. The applied research component of the DIL will be highlighted with vignettes of the responses to recent COVID-10 and Mpox outbreaks.

"Therapeutic antibody development for RNA viruses of pandemic potential"

Rebekah Honce, PhD
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The emergence and re-emergence of RNA viruses remains a critical threat to global health. However, few therapeutic or prevention strategies exist to combat emerging viruses. Further, key reagents needed to study these high-risk priority pathogens are limited. Here, we exploit a new sequencing technology to define the plasmablast-encoded antibody repertoire in patients during acute viral infection with the goal to develop broadly neutralizing antibodies for therapeutic application. Our efforts focused on two viral families of high clinical significance, *Coronaviridae* and *Hantaviridae*, and enrolled patients who exhibited a range of disease severity. Longitudinal patient samples were evaluated by analyzing expressed immunoglobulin (Ig) sequences from isolated blood plasmablasts. We sequenced natively paired antibody (Ab) sequences from our cohorts of COVID-19 or hantavirus cardiopulmonary syndrome (HCPS) patients and synthesized monoclonal (m)Abs corresponding to more than 500 unique Ab lineages. These candidate mAbs were screened for *in vitro* neutralization of authentic virus, binding specificity, and, for a subset of our most promising lead molecules, *in vivo* protection. Of interest, several candidate Abs neutralized SARS-CoV-2 infectivity *in vitro*, provided complete protection against lethal SARS-CoV-2 challenge in K18 hACE2 transgenic mice, and displayed particularly potent *in vitro* neutralization activity across a panel of SARS-CoV-2 variants of concern. Some of these candidates also broadly cross-neutralized SARS -CoV-1 or MERS. We also identified candidate mAbs that neutralize Andes orthohantavirus (ANDV) *in vitro*. Notably, we tested how conversion of our candidate IgG1 mAbs to multivalent IgM mAbs might impact neutralization potency and observed that multivalency consistently improves mAb potency. Beyond therapeutic mAb discovery, our work has also uncovered mAb candidates recognizing other viral proteins beyond the envelope glycoprotein, including several structural proteins. Our plasmablast repertoire sequences likely encode additional mAbs that could recognize the rest of the viral proteomes of these viruses. In summary, the antibody repertoire characterization methods described here can be a resource for the discovery of candidate antibodies reactive across viral pathogen proteomes for use in cellular virology studies, diagnostics, and the development of novel therapeutics for protection against emerging pathogens of concern.

Authors Index

CS – Corporate Speaker.....P# - Poster Number

KS - Keynote Speaker .....S - Speaker

O - Oral Poster Presentation ..... P - Page

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Albany Medical College
Conference Safety Plan

Commitment to Safety

Albany Medical College (AMC), part of the Albany Med Health System (AMHS), is committed to providing a safe environment for this conference free of harassment, discrimination and retaliation. As a conference organizer and the recipient of an NIH R13 award supporting this conference, AMC is required to provide this Conference Safety Plan.

Conference Code of Conduct

It is expected that all conference participants, whether or not affiliated with AMC and regardless of their role, will conduct themselves with integrity, in a manner that enables everyone to work and learn with security and dignity, free from unwelcome, insulting, degrading or exploitive treatment or harassment. Potential violations of this Conference Code of Conduct should be made using one of the confidential channels set forth below.

Some examples of harassing, discriminatory and retaliatory behaviors include:

- Unwelcome sexual advances, requests for sexual favors, touching or impeding movements.
- Unwelcome comments or behavior in any medium related to an individual's race, ethnicity, color, national origin, sex, disability, religion, age, gender, gender identity, disability, height, marital status, political persuasion, sexual orientation, veteran status, or weight.
- Unwelcome teasing, joking, or flirting based on actual or perceived gender identity, gender expression, or sexual identity/orientation.
- Threats or insinuations, either explicit or implicit, that an individual's refusal to submit to or acquiesce in sexual advances or sexual conduct will adversely affect their education, employment, evaluation, wages, advancement, assigned duties, benefits or any other aspect of education, employment or career advancement.

Reporting Channels for Questions, Concerns & Complaints

Any conference participant who believes they have been subjected to conduct in violation of the Conference Code of Conduct or applicable policies, or who becomes aware of such conduct, is strongly encouraged to report it using one of the following confidential reporting channels:

- In an emergency, call 911
- Conference Organizing Committee members (Dawn Bellville, Katherine MacNamara and Michael Robek) will be available at the registration table during active conference hours.
- Title IX Deputy Coordinator and Chief Compliance Officer Noel Hogan: (518) 264-4692 or hoganc@amc.edu
- Director of Hospital Regulatory Affairs and Section 504 Coordinator Kara Ritschdorff: (518) 262-3577; TDD or State Relay Number: (518) 262-3756
- Confidential AMC TiPP Line (518) 264-8477 (anonymous optional)
- Online at: https://amc.cqs.symplr.com/Portal/CreateForm/450012 (anonymous optional)
- Email AMC Corporate Compliance at Compliance@amc.edu

- Call AMC Human Resources at (518) 262-8414
- For non-emergencies only, contact Cooperstown NY Police Department: (607) 547-2500
  22 Main Street, basement of village library, at Fair Street entrance

Individuals may also notify NIH (https://public.era.nih.gov/shape/public/notificationForm.era or (301) 480-6701 or file a complaint with HHS Office of Civil Rights at the address below related to harassment, including sexual harassment, discrimination, and other forms of inappropriate conduct at NIH-supported conferences.

U.S. Department of Health and Human Services
Office of Civil Rights
200 Independence Avenue, SW
Room 509F, HHH Building
Washington, D.C. 20201
1-800-368-1019, 800-537-7697 (TDD)

https://www.hhs.gov/ocr/complaints/index.html

http://www.hhs.gov/ocr/office/file/index.html

Filing a complaint with AMC is not required before filing a complaint of discrimination with HHS OCR, and seeking assistance from AMC in no way prohibits filing complaints with HHS OCR.

How AMC Responds to Reports

Reports will be referred promptly to the appropriate AMC office for investigation, which includes the assessment of allegations in light of evidence collected, in accordance with the applicable policy and procedure. The confidentiality of individuals reporting possible instances of noncompliance will be protected to the extent possible. Upon completion of an investigation, AMC students and employees found to have engaged in acts of harassment, discrimination or retaliation will be promptly disciplined. If circumstances warrant, discipline for AMC students or employees may include suspension, expulsion, or termination. Consequences may be pursued related to individuals who are not AMC students or employees, which may include ineligibility to participate in future Conferences organized by AMC.

Individuals making reports in good faith will not be retaliated against for having made the report regardless of whether noncompliant acts have occurred. Acts of retaliation or intimidation resulting from involvement in an investigation of potential instances of noncompliance are prohibited. All suspected acts of retaliation, intimidation, or other forms of harassment should be reported immediately to AMC Corporate Compliance.

AMC's Student Non-Discrimination and Harassment Policy and Complaint Procedure provides the grievance process for complaints alleging sexual harassment as defined by Title IX, as well as prohibited relationship violence, sexual misconduct, stalking and retaliation. Albany Med's Personal Conduct/Harassment policy provides the grievance procedure for complaints alleging all other forms of discrimination, harassment, and retaliation. AMC is responsible for receiving reports of discrimination, harassment or retaliation, and facilitating the filing of complaints under these policies, connecting affected individuals with supportive measures, accommodations, interim measures and other assistive resources, and for conducting investigations of alleged violations of both policies.

Additional Online Resources

Sexual harassment policy for New York State employers:

https://www.ny.gov/sites/default/files/atoms/files/StatewideSexualHarassment_PreventionPolicy.pdf

New York State human rights law:

https://dhr.ny.gov/system/files/documents/2023/06/human-rights-law-printable.pdf

U.S. E.E.O.C. harassment:

https://www.eeoc.gov/harassment

U.S. E.E.O.C. discrimination:

https://www.eeoc.gov/discrimination-type

NIH Supporting a Safe and Respectful Workplace at Institutions that Receive NIH Funding:

https://grants.nih.gov/grants/policy/harassment.htm

U.S. Department of Education Harassment Prevention Resources:

https://www2.ed.gov/about/offices/list/ocr/frontpage/pro-students/har-resources.html

Thank you for your cooperation with this Conference Safety Plan.

Enjoy the Conference!