RUTGERS HEALTH
Institute for Infectious and Inflammatory Diseases

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Immune and Metabolic Responses to Pathogens

Effective immune responses are critical for clearing pathogens and maintaining health. Infectious diseases—including those caused by pandemic viruses—combined with limited therapeutic options such as vaccines, have exposed significant gaps in our understanding of host defense mechanisms. These challenges underscore the urgent need to deepen our understanding of host-pathogen interactions and the factors that contribute to effective immunity and pathogen clearance.

In recent years, the emerging field of immunometabolism has revealed how cellular metabolic pathways shape the nature of immune responses. These insights have advanced our understanding of not only immune regulation in metabolic diseases and cancer, but also of host defense mechanisms across a wide range of infectious diseases.

This symposium will bring together internationally renowned experts with diverse perspectives to share recent advances at the intersection of immunity, metabolism, and microbiology. By highlighting notable findings and fostering interdisciplinary dialogue, this event aims to support collaborative efforts to promote health.

Rutgers, The State University of New Jersey

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Guest Speakers

Maxim Artyomov, PhD

Dr. Artyomov is a Systems Immunologist, focused on investigating complex immune phenotypes by integrating traditional experimental techniques with high-throughput data generation and analysis. I am currently an Alumni Endowed Professor at the Department of Pathology & Immunology and have been working at Washington University in St. Louis since 2012.

In the context of immunometabolism, we discovered and validated novel metabolic mechanisms controlling macrophage polarization as well as provided a comprehensive description of the global metabo-transcriptional rewiring (Immunity, 2015, e.g. aspartate-argininosuccinate shunt in proinflammatory macrophages, roles of glutamine and UDP-GLcNAc in alternative activation). We then followed up to discover the immunoregulatory properties of itaconate and describe its inhibition of Sdh, a natural metabolite produced by activating macrophages (Cell Metabolism, 2016; Nature, 2018; J Exp Med, 2018; Nature Review Immunology, 2019; Nature Metabolism, 2020; Cell Reports, 2021). In our latest work, we report novel itaconate phenotypes and describe molecular mechanism of itaconate's regulatory action (Nature Metabolism, 2025). We also develop computational tools for the analysis of metabolic networks (Nuc Acid Res, 2016; Cell Reports, 2023).

In parallel, we applied a systems biology approach to understanding the aging of the immune system in both mice and humans and defined a distinct subpopulation of age-associated GZMK+ CD8 T-cells that is dominant in old mice and is characterized by exhaustion markers (Immunity, 2021). Human aging has been further detailed in our massive profiling of PBMCs from ~250 donors (Nat Aging, 2021a). In a separate work, we have provided comprehensive multiomic characterization of the classical human monocytes and defined new DNA methylation signatures associated with human aging (Nat Aging, 2021b). We have summarized state of the field of the immune aging in the recent review titled 'Immune aging at single-cell resolution' (Nat Rev Imm, 2021). Next, we have dissected immune aging in healthy blood by profiling ~300 samples across all ages using scRNA-sequencing (Immunity, 2023). In most recent work (Immunity, 2024), we have dissected aging in naïve T cells and identified markers of human recent thymic emigrant cells.

Roi Avraham, PhD

Born in Jerusalem, Roi Avraham completed a BSc in computer science and management at Tel Aviv University in 2001. He earned his MSc magna cum laude in neuroscience there in 2006. He completed a PhD in biological regulation with Prof. Yosef Yarden at the Weizmann Institute of Science in 2011, followed by a postdoctoral fellowship at the Broad Institute of MIT and Harvard. He joined the Weizmann Institute's Department of Immunology and Regenerative Biology in May 2016. He received the prestigious scientific council prize and became an associate professor in 2023.

Aimee Beaulieu, PhD

Dr. Aimee Beaulieu is an Assistant Professor in the Department of Microbiology, Biochemistry, and Molecular Genetics at Rutgers New Jersey Medical School, and a faculty member of the Center for Immunity and Inflammation. She holds a PhD from Weill Cornell Graduate School of Medical Sciences and a BA from Colgate University. Dr. Beaulieu's research focuses on understanding the molecular and cellular signals that regulate immune responses, particularly those involving Natural Killer (NK) cells and "helper" innate lymphoid cells (ILC1s). Her lab employs a broad range of genetic, cellular, and molecular tools to explore novel pathways contributing to NK cell development, differentiation, and effector responses in steady-state conditions and during infection, pregnancy, injury, and cancer.

Ongoing projects in her lab include investigating molecular pathways that regulate antigen-specific effector and memory responses by NK cells during viral infection, differentiation and function of unique tissue-resident NK cell and ILC1 populations in tissues such as the uterus and skin, anti-tumor responses by NK cells, and NK cell-mediated modulation of other immune and non-immune cell types in infected or inflamed tissues. In addition to her research, Dr. Beaulieu mentors early-career investigators, contributing to the development of immune-based therapies.

Theresa L. Chang, PhD

Dr. Theresa L Chang is Principal Investigator at Public Health Research Institute and Professor of Microbiology, Biochemistry and Molecular Genetics at Rutgers, New Jersey Medical School. She received her BS in Microbiology from Soochow University, Taiwan and her MS in virology from Auburn University. She obtained her PhD in virology from New York University and did her postdoctoral trainings on RNA-protein interactions and cancer cell signaling at Yale University and Aaron Diamond AIDS Research Center. Her main research interest is to understand mucosal immune and microbial determinants important for infection, pathogenesis and disease progression. Her research has been continuously supported by NIH since 2005. She was a full member in the NIH study sections and continues to serve as an ad hoc reviewer.

Brian Daniels, PhD

Dr. Daniels is an assistant professor in the Department of Cell Biology and Neuroscience at Rutgers University, New Brunswick. The goal of his laboratory is to define basic mechanisms of neuroimmune crosstalk during neurologic disease states, with a particular emphasis on CNS viral infection.

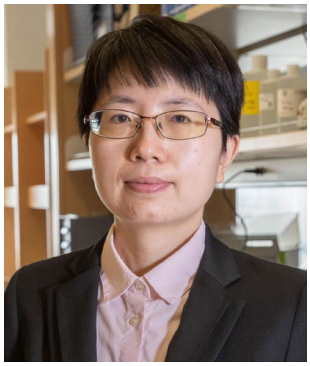
William Gause, PhD

Dr. William C. Gause is a distinguished immunologist and the Senior Associate Dean for Research at Rutgers New Jersey Medical School. He is also the Director of the Institute for Infectious & Inflammatory Diseases (i3D) and the Center for Immunity & Inflammation. Dr. Gause received his PhD in Immunology from Cornell University and completed postdoctoral work at the National Institutes of Health (NIH).

Prior to joining Rutgers in 2004, Dr. Gause held faculty positions at the Uniformed Services University of the Health Sciences, where he also served as Vice Chair of the Department of Microbiology and Immunology. At Rutgers, Dr. Gause has led significant research initiatives focused on immune responses to parasitic infections, particularly through the study of macrophage function during type 2 immune responses. His research, which has been continuously funded by the NIH since 1991, has resulted in over 100 publications in leading scientific journals. He has served on NIH study sections, editorial boards, and has organized symposia at major conferences. Dr. Gause is a Fellow of the American Association for the Advancement of Science (AAAS) and has received multiple awards for his contributions to biomedical research and education. Dr. Gause's work continues to influence the field of immunology, particularly in the areas of inflammation and immune resistance.

Bobby Brooke Herrera, PhD

Dr. Bobby Brooke Herrera is a scientist-entrepreneur working at the intersection of immunology, diagnostics, and global health. He earned his PhD in Biological Sciences in Public Health from Harvard University and completed postdoctoral training at Harvard Medical School. He later co-founded a company based at MIT's The Engine, where he led groundbreaking work on rapid diagnostics for infectious diseases—efforts that earned him a spot on the Forbes 30 Under 30 list in Healthcare. Now a faculty member at Rutgers Robert Wood Johnson Medical School, Dr. Herrera leads a research team focused on decoding and directing adaptive immunity. By developing field-deployable diagnostic platforms and engineering T cell–targeted immunogens, his lab aims to transform how we detect, understand, and ultimately prevent emerging infectious threats.



Pingping Hou, PhD

Dr. Pingping Hou is an Assistant Professor in the Department of Microbiology, Biochemistry, and Molecular Genetics and the Center for Cell Signaling. The Hou Lab investigates the molecular mechanisms of KRAS-targeted therapy resistance in pancreatic cancer, with a particular focus on the interplay between cancer cells and the tumor microenvironment. Additionally, the lab develops innovative myeloid cell-based therapies for solid tumors.

Chinglin Hsieh, PhD

Dr. Ching-Lin Hsieh currently serves as the Head of Structure-based Antigen Design in Sanofi Vaccine R&D. He obtained his BS and MS degrees from National Taiwan University, followed by a PhD training in Comparative Biomedical Sciences from Cornell University under the guidance of Dr. Yung-Fu Chang. Subsequently, Dr. Hsieh conducted his postdoctoral research at the University of Texas at Austin in Dr. McLellan's laboratory, where he specialized in structural biology techniques such as X-ray crystallography and cryo-electron microscopy to elucidate viral glycoprotein fusion process and antibody-mediated virus neutralization mechanisms. His expertise lies in utilizing atomic-level structural information of viral glycoproteins to inform rational vaccine antigen design with enhanced immunogenicity. Since joining Sanofi in 2022, Dr. Hsieh has continued his research on class I viral fusion proteins while expanding his expertise to bacterial antigens, with the overarching goal of developing superior vaccine antigens to combat infectious diseases.

Jack Hsu, PhD

Dr. Jack Chun-Chieh Hsu is an Assistant Professor in the Department of Medicine and a member of the Center for Virus-Host-Innate Immunity (CVHII) at Rutgers NJMS. He earned his BS and MS degrees from National Taiwan University and completed his PhD in Biochemistry and RNA Biology at Duke University under the mentorship of Dr. Christopher Nicchitta. Dr. Hsu then pursued postdoctoral research at Yale University in Dr. Peter Cresswell's laboratory, focusing on virus-host interactions and innate antiviral responses. In Summer 2023, Dr. Hsu joined Rutgers NJMS, where he established his own lab. His research investigates RNA translation and modifications in the context of viral infections, aiming to understand how viruses manipulate host translation machinery and how type I interferon responses restrict viral replication. The lab's work contributes to the development of novel antiviral strategies and broad-spectrum biomarkers.

Estela Jacinto, PhD

Dr. Estela Jacinto is a Professor in the Department of Biochemistry and Molecular Biology at Rutgers Robert Wood Johnson Medical School in New Jersey. She earned her B.S. in Zoology from the University of the Philippines in 1986 and her PhD in Biomedical Sciences from the University of California, San Diego, in 1997, where she studied the role of MAP kinases in T cell signaling in Michael Karin's lab. Dr. Jacinto was a Cancer Research Institute Postdoctoral Fellow at the Biozentrum, University of Basel, Switzerland, in the lab of Michael N. Hall, a pioneer in mTOR research, where she contributed to identifying and characterizing the mTOR protein complexes. Her research focuses on understanding how nutrients influence cell signaling and metabolism to regulate T cell development, with the aim of enhancing immunity through more effective dietary strategies.

Jason Kaelber, PhD
Dr. Kaelber trained under Colin Parrish at Cornell and obtained his PhD in Molecular Virology under Wah Chiu. He is the director of the Rutgers CryoEM & Nanoimaging Facility since 2017 and 3DEM Advisor to the RCSB Protein Data Bank since 2024. Dr. Kaelber is an Associate Research Professor at Rutgers where his lab focuses on parvovirus structure/function relationships and virus engineering.

Ryan Langlois, PhD
Ryan received his BS in biology from the University of Wisconsin - Stevens Point in 2005. He then earned a PhD with Kevin Legge in 2010 at the University of Iowa studying dendritic cell - T cell interactions. He then moved to NYC working with Ben tenOever as a postdoc at Mount Sinai. Here he combined molecular virology and immunology to build new model systems to study virus host interactions. In 2014 Ryan started his independent position at the University of Minnesota where he works on virus-host interactions and more recently developing models to study cross species virus replication.

Maudry Laurent-Rolle, MD, PhD
Maudry Laurent-Rolle is an Assistant Professor in the Department of Internal Medicine, Department of Microbial Pathogenesis and Center for Infection and Immunity at Yale University. She completed her MD and PhD at the Icahn School of Medicine at Mount Sinai with Dr. Adolfo García-Sastre where she studied the molecular mechanisms by which flaviviruses inhibit host innate immune responses. Dr. Laurent-Rolle went on to do her residency training in Internal Medicine at Albert Einstein/Montefiore Medical Center. Subsequently, she joined the Infectious Diseases Fellowship program at Yale New Haven Hospital and did her postdoctoral research studies under the mentorship of Dr. Peter Cresswell where she investigated the molecular mechanisms that host antiviral proteins like viperin and CMPK2 use to restrict viral replication. Dr. Laurent-Rolle joined the faculty at Yale University in 2020. The Laurent-Rolle lab is interested in understanding how host responses to viruses enhance or prevent diseases and how pathogenic viruses evade the host immune response.

Ai Ing Lim, PhD
Dr. Lim is an Assistant Professor in the Department of Molecular Biology at Princeton University. Prior to joining Princeton, she received her scientific training at the Institut Pasteur and the NIH. Her lab studies how maternal immunity adapts during reproduction and how maternal environmental exposures influence the development of the offspring's immune system. The motivation to study maternal-offspring immune crosstalk is threefold: (1) to understand and mitigate pregnancy complications, with the goal of improving women's health; (2) to uncover early drivers of immune disorders and improve immune trajectories in future generations; and (3) to explore how interactions with microbes and other environmental cues shape the evolution and function of the immune system.

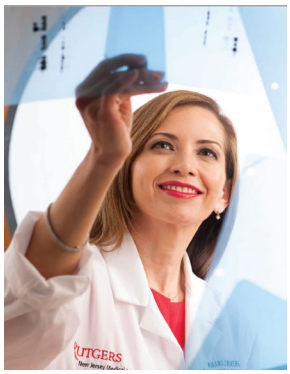
Shan-Lu Liu, MD
Dr. Liu directs the Viruses and Emerging Pathogens Program at OSU's Infectious Diseases Institute and serves as Associate Director of the Center for Retrovirus Research. He is an Editor of Journal of Virology, a Guest Editor for mBio, PNAS, and PLoS Pathogens, and an Associate Editor of Viruses, in addition to serving on multiple editorial boards.

Cindy Meyer, PhD
Dr Meyer graduated in Biochemistry from the University of Leipzig (Germany) in 2006, and completed her PhD at the University of Hamburg (Germany) in 2009, focusing on the in vitro evolution of RNA aptamers and their therapeutic potential. In 2012, she joined Tom Tuschl's lab at The Rockefeller University with a fellowship from the German Academic Exchange Service (DAAD), where she focused on the functional characterization of human RNA-binding proteins involved in ribosome-associated quality control and cellular stress response pathways. With the onset of the SARS-CoV-2 pandemic, Cindy shifted her research to developing small molecule inhibitors targeting essential viral enzymes, including those involved in viral RNA replication and capping.

Veronika Miskolci, PhD
Veronika earned her PhD at Albert Einstein College of Medicine. Her thesis focused on the coordination of Rho GTPase signaling during macrophage chemotaxis using FRET-based biosensors under the mentorship of Dianne Cox and Louis Hodgson. She went on to the University of Wisconsin-Madison to complete her postdoctoral training in the laboratory of Anna Huttenlocher, where she studied the regulation of innate immunity during tissue repair using larval zebrafish models of sterile injury.

Edward Pearce, PhD
Edward Pearce received his PhD for research performed at the National Institute for Medical Research in London, and did his postdoctoral training at the National Institutes of Allergy and Infectious Diseases in Bethesda. His first independent position was as an Assistant Professor at Cornell University, and he has subsequently held faculty positions at the University of Pennsylvania, The Trudeau Institute, Washington University in St. Louis, The Max Planck Institute of Immunobiology and Epigenetics and The University of Freiburg, and is currently a Bloomberg Distinguished Professor of Immunobiology at the Bloomberg Kimmel Institute of Cancer Immunotherapy at Johns Hopkins University. He trained as an immunologist and parasitologist, and has for many years focused on immunity to parasitic infections, with a particular emphasis on chronic infections caused by parasitic helminths. While at the University of Pennsylvania he became interested in the metabolic processes that underlie immune cell biology and has since been actively engaged in research that explores the importance of metabolic transitions in immune cell activation, function and fate. Work in his laboratory encompasses the exploration of immune cell function during infection, cancer and autoimmunity. The aspirational long-term goal of his group is to be able to provide insights that allow the development of approaches to mitigate human disease.

Ricardo Rajsbaum, PhD
Ricardo Rajsbaum, PhD is an Associate Professor in the Department of Medicine, and the director of the Center for Virus-Host-Innate Immunity (CVHII) at Rutgers New Jersey Medical School. Dr. Rajsbaum obtained his PhD at the MRC-NIMR, London, UK, and completed his postdoctoral training at Mount Sinai School of Medicine, New York. Dr Rajsbaum started his independent research program as an Assistant Professor at UTMB, Galveston, Texas. Dr. Rajsbaum's research interests include innate immune regulation, Pattern Recognition Receptor (PRR) signaling, regulation and function of type-I Interferons, virus–host interactions, and viral evasion of antiviral immunity, with a specific focus on the role of the host ubiquitin system. Research in the lab centers on the molecular mechanisms by which E3-Ubiquitin ligases promote resistance or susceptibility to virus infections.



Amariliz Rivera, PhD

Dr. Rivera is a tenured, associate professor at Rutgers New Jersey Medical School in Newark, NJ. She is a 2023-2025 Distinguished Lecturer for the American Society of Microbiology and president-elect to the Medical Mycological Society of the Americas (MMSA). Amariliz Rivera received her B.S from the University of Puerto Rico-Mayaguez campus and her PhD from Rutgers-Robert Wood Johnson Medical School. She did her postdoctoral training at MSKCC where she began the abiding theme of her research: achieving a better understanding of how the immune system fights fungal infections. After her training, she joined the Center of Immunity and Inflammation faculty in 2010. Dr. Rivera has been serving as Associate Director of CII since 2020 and as associate director of NJMS MD.PhD. program since 2024. Her work through the years has delineated fungus-specific CD4 T cell responses and monocyte-, monocyte-derived dendritic cell- and neutrophil-mediated innate antifungal immune responses in the context of pulmonary fungal disease. Her research is currently supported by NIAID and the Burroughs Wellcome Fund Investigators in the Pathogenesis of Infectious Disease award.

Padmini Salgame, PhD

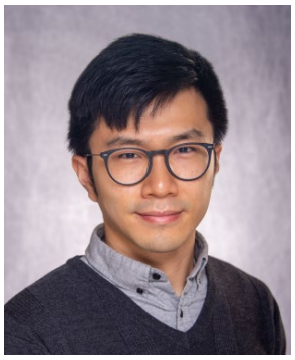
Dr. Salgame is a Professor in the Department of Medicine, Center for Emerging and Re-Emerging Pathogens, Rutgers University. She is Co-Director of the MD/PhD Program and Associate Director of The Public Health Research Institute (PHRI). Her research program employs human and animal models to study immunity to tuberculosis and the impact of *Mycobacterium tuberculosis* strain variation on transmission and infection, and to identify host biomarkers across the disease spectrum. She collaborates widely with researchers from the U.S., Brazil, India, and Uganda. Dr. Salgame is an Editor for *Infection and Immunity* and an Associate Editor for *PLOS Pathogens,* and she also manages a range of science teaching and training programs.

Henrique Serezani, PhD

Dr. Serezani joined the faculty in the Department of Medicine in 2016 as an assistant professor in the Division of Infectious Diseases, becoming an associate professor in 2020. Since 2016, he has published over 80 research articles, making a significant impact both in the field of research and education and in the Department and School of Medicine. Most of Dr. Serezani's manuscripts have been published in high-impact journals such as Science Signaling, PNAS, J. Clinical Investigation, PLoS Pathogens, Diabetes, and the Journal of Immunology. He has been funded by the NIH for over 15 years. He has trained seven postdocs who currently hold faculty positions in different institutions. Also, 8 graduate students successfully defended their PhD thesis under his mentorship. Dr. Serezani has established a robust line of research in inflammation/immunity and host defense and has extended this research into other areas of interest, such as metabolic disease and sepsis. Dr. Serezani's national reputation has been growing over the last few years with invitations to present his research, requests to join editorial boards (Diabetes, J. Leukocyte Biology and Immunohorizons), and invitations to both ad hoc and as a standing member in different NIH study sections. He is an active member of the American Association of Immunologists and the American Diabetes Association, as well as a council member of the Society for Leukocyte Biology. Dr. Serezani is also a leader in different areas within VUMC and VU. He is the associate director of the Vanderbilt Center for Immunobiology and co-director of the Initiative to Maximize Student Development at Vanderbilt (IMSD-V) – a T32-funded program that aims to recruit and mentor underrepresented students in graduate programs. Outside the lab, he enjoys watching his daughters play soccer, basketball and doing taekwondo with them.

George Yap, PhD

George S. Yap is Professor of Medicine and Member of the Center for Immunity and Inflammation at New Jersey Medical School. A graduate of the University of the Philippines and of McGill University in Canada, Dr. Yap has been studying host resistance and disease tolerance mechanisms during parasitic diseases for the past three decades. He is an elected Fellow of the American Academy of Microbiology.



Roy Wong, PhD

Dr. Wong received his PhD at the University of Hong Kong where he studied the role of coronavirus proteins in perturbing innate immune responses. He then moved to Iowa and joined Dr. Stanley Perlman's lab for his postdoctoral training and research on coronavirus pathogenesis. During his postdoctoral training, Dr. Wong studied and characterized the Spike mutations found in human and camel MERS-CoV isolates in relation to pathogenesis. At the time of the COVID-19 pandemic, Dr. Wong was involved in developing several mouse models for SARS-CoV-2 infection that have been widely used by the scientific community. He also isolated a mouse-adapted strain of SARS-CoV-2 that causes severe disease in mice as a pathogenic model for COVID-19 for studying SARS-CoV-2 pathogenesis and vaccine and antiviral development. Dr. Wong joined the Center for Virus-Host-Innate Immunity (CVHII) at Rutgers NJMS as an Assistant Professor and Chancellor Scholar in the Department of Microbiology, Biochemistry and Molecular Genetics in the Fall of 2023. The Wong lab's research focus on coronavirus pathogenesis will be addressed by understanding 1) CoV protein functions in relation to pathogenesis; 2) Immunopathogenesis caused by CoV infection; 3) Development of animal models for studying CoV pathogenesis. The lab uses various virology and immunology approaches to investigate virus- and immune (host)-mediated pathogenesis in various model systems.

Tania Wong, PhD

Dr. Tania Wong is an Assistant Professor and Chancellor Scholar in the Department of Microbiology, Biochemistry & Molecular Genetics at Rutgers New Jersey Medical School. Her laboratory, based within the Center for Immunity and Inflammation (CII), investigates host-pathogen interactions, with a particular focus on how airway pathogens such as *Staphylococcus aureus* and *Klebsiella pneumoniae* manipulate host metabolic responses to evade immune clearance.

Dr. Wong's prior work demonstrated that *K. pneumoniae* associated with prolonged infections activates host metabolic pathways that fuel mitochondrial oxidative phosphorylation (OXPHOS), creating a milieu that fosters an immune response permissive of infection. Her current research expands on these findings by exploring how specific metabolic pathways and/or metabolites influence immunity and infection outcomes. She employs metabolic and dietary interventions to promote effective immune responses and pathogen clearance. In parallel, her lab investigates how immune-signaling metabolites that accumulate during infection directly affect bacterial pathogens—potentially driving the selection of strains better adapted to survive in oxidant-rich environments.

Dr. Wong received her PhD from the University of Melbourne, Australia, and completed her postdoctoral training at Columbia University in New York. She is a recipient of the NIH/NHLBI K99/R00 Pathway to Independence Award and serves as the Scientific Director of a new spatial metabolomics facility at the CII.

Oral Presentations

Maxim Artyomov, PhD, Washington University School of Medicine
Talk: Molecular mechanism of itaconate's immunoregulatory action

Roi Avraham, PhD, Weizmann Institute of Science
Talk: Hypoxia-Driven Immune Remodeling During Typhoid Gut Invasion Shapes Infection Outcome

Aimee Beaulieu, PhD, Rutgers University
Talk: Regulation of liver ILC1 function and metabolism

Theresa L. Chang, PhD, Rutgers University
Talk: Role of IFNe in viral infection and cancer

Brian Daniels, PhD, Rutgers University
Talk: Asymptomatic adult Zika virus infection incurs long term neurologic consequences

William Gause, PhD, Rutgers University
Talk: Neutrophil heterogeneity and reprogramming in a type 2 immune response

Bobby Brooke Herrera, PhD, Rutgers University
Talk: Uncovering Age-Specific Immune Barriers to Protection and Vaccine Efficacy Against La Crosse Virus Encephalitis

Pingping Hou, PhD, Rutgers University
Talk: The Role of Macrophages in Resistance to KRAS-Targeted Therapy

Chinglin Hsieh, PhD, Sanofi
Talk: Prefusion stabilization of class I viral fusion proteins

Jack Hsu, PhD, Rutgers University
Talk: Coronavirus NSP14 protein induces RNA internal m^7G modification and alternative splicing via host transcription machinery.

Estela Jacinto, PhD, Rutgers University
Talk: mTOR signaling and hexosamine biosynthesis: supply and demand dynamics during early T cell development

Jason Kaelber, PhD, Rutgers University
Talk: Uncovering a pandemic's cause, and gene delivery mechanisms, by virus nanoimaging

Ryan Langlois, PhD, University of Minnesota
Talk: Evaluating the barriers to cross-species virus infections

Maudry Laurent-Rolle, MD, PhD, Yale University School of Medicine
Talk: Mitochondrial Dynamics as a Nexus for Alphavirus Replication Strategies

Ai Ing Lim, PhD, Princeton University
Talk: Maternal-Offspring Immune Partnership

Shan-Lu Liu, MD, PhD, The Ohio State University
Talk: Immune Evasion and Cell-to-Cell Transmission of SARS-CoV-2

Cindy Meyer, PhD, The Rockefeller University
Talk: The SARS-CoV-2 RNA cap methyltransferase NSP14: Exploring new targets for antiviral drug development

Veronika Miskolci, PhD, Rutgers University
Talk: Immunoresponsive gene 1 supports collagen remodeling following sterile injury

Edward Pearce, PhD, Johns Hopkins University
Talk: CSF2 induced metabolic changes underlying dendritic cell development from monocytes

Ricardo Rajsbaum, PhD, Rutgers University
Talk: Regulation of Immunity and Virus Replication by Host Posttranslational Modifications, and Novel Therapeutic Opportunities

Amariliz Rivera, PhD, Rutgers University
Talk: Neutrophils and interferons meet at unexpected places

Padmini Salgame, PhD, Rutgers University
Talk: Uncovering bacterial and host interactions driving heterogeneity in M. tuberculosis transmission and infection outcome

Henrique Serezani, PhD, Vanderbilt Institute for Infection, Immunology and Inflammation
Talk: Metabolic memory, epigenetics, and increased susceptibility to sepsis in diabetic conditions.

George Yap, PhD, Rutgers University
Talk: Immune control of the metabolic stress response to infection

Roy Wong, PhD, Rutgers University
Talk: Contrasting roles of MERS-CoV and SARS-CoV-2 internal proteins in pathogenesis

Tania Wong, PhD, Rutgers University
Talk: Diet-mediated immunometabolic regulation promotes K. pneumoniae airway clearance

POSTERS

1. Kazi Afreen - The role of the deubiquitinase USP11 during Ebola Virus Infection
2. Reem Alatrash - Immunodominant structural proteins Gc and N drive T cell-mediated protection against La Crosse virus
3. Juan Angel - Astrocytic p53 Orchestrates Flavivirus Neurotropism via Metabolic and Glycosylation Reprogramming
4. Suheyla Batirbek - Helminth infection favors persistent and proliferating alternatively activated neutrophils in the lung
5. Padmanava Behera - The Host Mannose Receptor Type C1 (MRC1) Acts as an Ebola Virus Entry Factor
6. Krupa Chavan - Investigating the contributions of hematopoietic stem and progenitor cells to antihelminth immunity and host protection
7. Garam Choi - Exploring the effect of diet on Bifidobacterium colonization in the gut using a gnotobiotic mouse model
8. Gbenga Dairo - Metabolomic signatures of the steroid biosynthesis driving sex differences in clinical asthma subtypes
9. Evan DaPrano - Asymptomatic adult Zika virus infection incurs long term neurological consequences
10. Nadia Domingo - Fibrinogen activates microglia through CD11b binding and regulates hypothermia in hyperinflammatory experimental cerebral malaria.
11. Ryan Fink - Identifying the Interactome of the key regulator, H2-O, in the MHC-II Antigen Presentation Pathway
12. Rebecca Francis - Differentiation pathway of Tbet+CD11c+ B cells influences their functional properties during acute viral infections
13. Corey Gallen - Role of CD103 in Reactivation and Maintenance of Small Intestine CD8+ Trm
14. Sebastian Gallon - Distinct Flavivirus Exposure Sequences Differentially Prime the Adaptive Immune Response
15. Komi Gdedande - In Vivo Contribution of IFN-γ^{+}IL-21^{+} Th1/Tfh Hybrid Cells in Germinal Center Dynamics for Protective Immunity Response
16. Lia Goodwin - M-CSF and GM-CSF hMDMS possess diferent functional activation states

17. Anjali Gupta - Investigating the dynamics and significance of epitranscriptomics in positive sense RNA viruses' genome

18. Chien-Hsin Huang - Uncovering the Role of Viperin-Derived ddhC in Influenza Pathogenesis

19. Vishal Karuppusamy - Zbtb20-expressing T cells as a potential regulator of cancer growth and multiple sclerosis

20. Pooja Kayala - Preferential reliance on glycolytic metabolism by liver ILC1s

21. Jisun Kim - TLR4–MyD88–NF-κB signaling promotes *Acinetobacter baumannii* clearance in the airway via neutrophil recruitment

22. Sarah Lahire - ZBTB22 regulates CD8+ T cell function and persistence in colorectal cancer

23. Giuseppina Marchesini Tovar - Long-chain fatty acid sensing by GPR132 regulates CD8+ T cell responses to infection

24. Eduard Marmut - Astrocytic RIPK3 exerts protective anti-inflammatory activity during viral encephalitis via induction of serpin protease inhibitors

25. Joaquin Moreno Contreras - The E3-Ubiquitin ligase TRIM6 and Unanchored Ubiquitin Promotes Damaging Neutrophilic Inflammation During Viral Infection via the PI3K-AKT Pathway

26. Janaki Ramya Namburu- Integrated DNA-methylome and Transcriptome Signatures Identify Disrupted Developmental Pathways by Prenatal Smoke Exposure

27. Yoatzin Penaflor-Tellez - Analysis of hosts protein ubiquitination during Ebolavirus infection.

28. Jessica Rappaport - Deciphering the Role of Zbtb20+ T-cells in the Development and Progression of Type 1 Diabetes

29. Alcina A Rodrigues- Role of G9a in NK cell proliferation and metabolism

30. Ensueno Saenz Altamirano - SARS-CoV-2 NSP14 Induces Internal m7G RNA Modifications and Alters Host Transcriptome

31. Natalia Teruel - Comparative Structural Evaluation of Ebola virus GP-Receptor Interactions Across Viral Species and Host Tropism Implications

32. James A. Tranos - Structure Based Drug Discovery Targeting Polymyxin Antibiotic Resistance

33. Abbey Warren - The Role of Post-Translational Modifications During Ebola virus Induced Immune Dysregulation

34. Hong Li - New Instrumentation and Services at the Center for Advanced Proteomics Research: Advancing Immune and Metabolic Research in Pathogen Responses

35. Lanbo Shi - Metabolic Reprogramming of Host Immunity During Tuberculosis: Pathways to Host-Directed Therapies

36. Alexandros Skouris- EGFR biased signaling in intestinal health and disease.

37. Eden Hirsh - Tfh cell regulation and the impact on B cells in viral infection

38. Paul Brennan - Characterization of the Pulmonary Arteriole as a Portal of Entry for Rapid Neutrophil Influx to *Coccidioides* Spore-Exposed Terminal Bronchioles.

39. Ridhima Wadhwa - IDO-dependent metabolic reprogramming promotes *K. pneumoniae* airway infection

Lecture

Molecular mechanism of itaconate's immunoregulatory action

Maxim Artyomov, PhD
Washington University School of Medicine

Itaconate is a unique metabolite produced in large amounts in myeloid cells upon stimulation by pathogen or damage-associated molecular patterns. Our lab initially identified it as an immunoregulatory metabolite with mild anti-inflammatory properties and ability to inhibit Sdh (Lampropoulou et al, 2016). Subsequent research from our lab using non-derivatized itaconate revealed its mild electrophilic properties (Bambouskova et al, 2018). We next identified that itaconate boosts type I interferon production (Swain et al, 2020), while it plays major suppressive role in the context of late inflammasome activation (Bambouskova et al, 2021). These discoveries shifted the understanding of itaconate from an immunosuppressive to an immunoregulatory metabolite.
In our latest work (Paulenda et al, 2025), we report major in vitro and in vivo interferon enhancing phenotypes for endogenous itaconate and show that immunoregulatory properties of itaconate, e.g. enhancement of type I interferon secretion, depends on inhibition of Peroxiredoxin 5 and on mitochondrial reactive oxygen species. We find that itaconate non-covalently inhibits Peroxiredoxin 5, leading to the modulation of mitochondrial peroxide in activating macrophages. Through genetic manipulation, we confirm that Peroxiredoxin 5 modulates type I interferon secretion in macrophages. The non-electrophilic itaconate mimetic 2-methylsuccinate inhibits Peroxiredoxin 5 and phenocopies immunoregulatory action of itaconate on type I interferon and inflammasome activation providing further support for a non-covalent inhibition of Peroxiredoxin 5 by itaconate. Our work establishes molecular mechanism of actions and biological rationale for predominantly immune specification of itaconate. Specifically, itaconate's production is conserved across species in innate-like immune cells and is triggered by various stimuli. Our work shows that itaconate enhances the effectiveness of reactive oxygen/nitrogen species, a key antimicrobial mechanism of innate immune cells, by inhibiting the natural antioxidant response.

Lecture

Hypoxia-Driven Immune Remodeling During Typhoid Gut Invasion Shapes Infection Outcome

Roi Avraham, PhD
Weizmann Institute of Science

Salmonella Typhi (*S.* Typhi), the causative agent of typhoid disease, remains a major public health concern. Owing to the human-restricted nature of *S.* Typhi, studies of typhoid pathogenesis in animal models are limited to a murine non-typhoidal pathogen. More recently, human challenge models have been conducted, providing insight into immune correlates of infection outcomes, which are still incompletely understood. We performed an integrated single-cell analysis of immune responses from the human *S.* Typhi challenge model and mouse model of typhoid disease, to associate biological mechanism with human infection outcome. Most prominent, we revealed immune subsets with a hypoxia-related signature in circulating immune cells from individuals that develop the disease in the human challenge model. This signature was also evident in the mouse model in activated macrophages

infiltrating into the Peyer's patches, but not during infection with a mutant strain impaired for gut invasion. Collectively, we revealed a hypoxia-related signature that links immune responses during bacterial invasion to increased risk of developing typhoid disease in humans, suggesting a possible causative role during the development of typhoid disease.

Lecture

Regulation of liver ILC1 function and metabolism

Aimee Beaulieu, PhD
Rutgers University

The liver is an important metabolic organ that is populated by unique liver-resident immune cells, including Type 1 "helper" innate lymphoid cells (ILC1s). Although ILC1s share many phenotypic and functional similarities with classic NK cells (cNKs), they are a separate lymphocyte lineage with non-redundant roles in memory responses to infectious and non-infectious antigens, tissue repair after liver injury, and control of liver metastases. Here, we investigate the unique regulatory signals and metabolic dependencies that inform liver ILC1 function at steady-state and during inflammation.

Lecture

Role of IFNe in viral infection and cancer

Theresa L. Chang, PhD
Rutgers University

Interferon epsilon (IFNe) is a unique type I interferon that shares only 37% amino acid homology with IFNa/b. Unlike IFNa/b, which is nearly undetectable at baseline, IFNe is constitutively expressed in epithelial cells of mucosal tissues including the female reproductive tract, where it regulates epithelial barrier integrity, tissue architecture, and immune cell function—all critical for host defense. We found that IFNe protects hosts against HIV and Zika infection through mechanisms distinct from IFNa. Interestingly, while IFNe expression can be induced in response to viral infection, SARS-CoV-2 suppresses IFNe in the olfactory epithelium. Beyond its anti-viral activity, IFNe has previously been reported to act as a tumor suppressor in ovarian cancer. However, our study reveals a contrasting role in cervical cancer. We found that IFNe is highly expressed in cervical cancer tissues and cell lines and promotes tumorigenesis. Knockdown of IFNe (IFNe KD) inhibited oxidative phosphorylation, cell proliferation, migration, colony formation, and tumor growth in xenograft models. IFNe depletion enhanced the sensitivity to staurosporine and cisplatin. RNAseq revealed that IFNe KD resulted in downregulation of NDUFA12, which encodes a subunit of mitochondrial Complex I in the electron transport chain. Re-expression of either NDUFA12 or IFNe in IFNe KD cells restored their tumorigenic capacity, including growth in xenograft models. Together, our findings uncover novel mechanisms of IFNe-mediated viral infection and tumorigenesis, highlighting a potential therapeutic target for prevention and treatment.

Lecture

Asymptomatic adult Zika virus infection incurs long term neurologic consequences

Brian Daniels, PhD
Rutgers University

Recent work has identified systemic viral infections as a risk factor for a variety of chronic neurologic syndromes. To date, much of the work linking viral pathogens to neurologic disease has focused on the impact of severe neuroinvasive infection and encephalitis. However, the potential neurologic consequences of mild or asymptomatic infections, which constitute the vast majority of human cases, are poorly understood. Here, we have adapted a model of systemic Zika virus infection in immunocompetent mice with a humanizing mutation in innate immune signaling. These mice experience an asymptomatic disease course similar to that of healthy adult humans infected with ZIKV. While ZIKV infection is rapidly cleared from the CNS, infected mice experience robust neuroinflammation, including recruitment of inflammatory leukocytes to the brain and the establishment of tissue resident memory T cells (TRMs) that persist for up to 1 year post infection. Multi-omic profiling demonstrates similarly long-lasting transcriptional and metabolic changes associated with neurodegeneration in resident CNS cell types. Machine learning-based behavioral profiling also demonstrates that these molecular alterations are associated with chronic changes to locomotor behavior. We further show that chronic neurologic impacts in our model appear to be driven by CD8 T cells, as depletion of these cells diminishes neuroinflammation and neuropathogenesis while producing only minor delays in virologic control. Our findings underscore the potential for chronic neurologic consequences following even asymptomatic viral infections and identify cytotoxic lymphocytes as key drivers of pathologic neuroinflammation in the postinfectious brain.

Lecture

Neutrophil heterogeneity and reprogramming in a type 2 immune response

William Gause, PhD
Rutgers University

Neutrophils are a granulocytic population of myeloid cells that have critical effector functions during infectious disease but are generally thought to be short-lived and nonproliferative with markedly limited activation states. In these studies, we directly compared lung neutrophil activation following infection with different groups of pathogens including bacteria, fungi, and helminths. Our results demonstrate considerable heterogeneity depending on the type of infectious agent. In contrast to bacterial and fungal infection, after helminth infection neutrophils expressed markers associated with characteristic type 2 responses, including IL-13, and unexpectedly upregulated genes associated with cell cycling and protein synthesis. Further studies showed reduced neutrophil cell death following helminth infection and increased proliferation, which was dependent on IL-4R signaling. This distinct subset of proliferating neutrophils expanded following helminth infection and was released from the endothelial niche to colocalize with invading parasites in the airways. These studies demonstrate a novel long-lived cycling phenotype for neutrophils following helminth infection.

Lecture

Uncovering Age-Specific Immune Barriers to Protection and Vaccine Efficacy Against La Crosse Virus Encephalitis

Bobby Brooke Herrera, PhD
Rutgers University

This talk focuses on La Crosse virus (LACV), the leading cause of pediatric arboviral encephalitis in the United States, for which no rapid diagnostics or licensed vaccines currently exist. We developed a highly sensitive reverse transcription recombinase polymerase amplification (RT-RPA) assay that enables early detection of LACV neuroinvasion in murine models, revealing that viral entry into the brain occurs one day earlier in weanling mice than in adults. This discovery prompted detailed investigations into age-specific immune responses, uncovering that adult mice mount robust Th1-skewed cytokine profiles, generate potent neutralizing antibodies, and exhibit polyfunctional cytotoxic $CD4^+$ and $CD8^+$ T cell responses, whereas weanling mice display Th2-biased cytokine patterns and impaired T cell effector function. These deficiencies correlated with poor viral control and increased mortality in younger animals. Building on these findings, we engineered a subunit vaccine incorporating the immunodominant glycoprotein and nucleocapsid proteins, which restored antiviral T cell responses and significantly improved survival in vaccinated weanlings. Ongoing mechanistic studies are interrogating the roles of regulatory T cells, PD-1 and CTLA-4 checkpoint pathways, and antigen-presenting cell dysfunction in shaping the immunological landscape of early life. Collectively, this work defines critical barriers to antiviral protection in young hosts and outlines a translational framework for developing age-targeted diagnostics and vaccines to prevent viral encephalitis in pediatric populations.

Lecture

The Role of Macrophages in Resistance to KRAS-Targeted Therapy

Pingping Hou, PhD
Rutgers University

KRAS-targeted therapy is essential for pancreatic cancer treatment. In this talk, we will discuss our previous and latest findings on how tumor-associated macrophages contribute to therapy resistance through interactions with cancer cells.

Lecture

Prefusion stabilization of class I viral fusion proteins

Ching-Lin Hsieh, PhD
Sanofi

Class I viral fusion proteins are critical for viral entry into host cells and undergo dramatic conformational changes from prefusion to postfusion states during the viral-host fusion process. Prefusion stabilization has emerged as a key strategy in vaccine development, as antibodies targeting

the prefusion conformation often exhibit superior neutralizing activity. However, the prefusion state is metastable and easily transitions into the postfusion state when expressed recombinantly in mammalian cells. Thus, we use structural information from atomic-resolution of viral fusion proteins to rationally guide prefusion-stabilized designs by using a variety of strategies, such as disulfide bonds, proline stabilization, charge optimization and cavity filling. Successful applications include respiratory syncytial virus and human metapneumovirus fusion proteins, influenza hemagglutinin, and SARS-CoV-2 spike protein, where prefusion-stabilized immunogens have demonstrated enhanced immunogenicity and protective efficacy in preclinical and clinical studies. This strategy represents a significant advancement in structure-based vaccine design against major human respiratory viruses.

Lecture

Coronavirus NSP14 protein induces RNA internal m7G modification and alternative splicing via host transcription machinery

Chun-Chieh Hsu, PhD
Rutgers University

Coronaviruses are a diverse family of positive-sense RNA viruses responsible for diseases ranging from the common cold to severe respiratory illnesses such as SARS, MERS, and COVID-19. Their large genomes and multifunctional proteins allow for complex interactions with host cellular machinery, facilitating replication and immune evasion. Among these viral proteins, the SARS-CoV-2 nonstructural protein 14 (NSP14) is known for its essential roles in viral RNA capping and proofreading. We found a previously unrecognized function of NSP14 in inducing internal N7-methylguanosine (m7G) modifications in host mRNA. We demonstrate that NSP14 induces internal m7G modification in host mRNAs via its guanine-N7-methyltransferase (N7-MTase) activity. Mechanistically, NSP14 catalyzes the methylation of GTP to form m7GTP, which is then incorporated into nascent transcripts by host RNA polymerase II. This non-canonical RNA modification is predominantly nuclear, enriched in mRNAs, and conserved across NSP14 homologs from human and animal coronaviruses. Notably, these internal m7G modifications alter host mRNA splicing patterns, promoting transcriptome-wide intron retention. Using RNA immunoprecipitation, in vitro transcription assays, and RNA-Seq, we show that NSP14-induced m7G plays an important role in alternative splicing. Using NSP14 mutants, N7-MTase inhibitors, or scavenging of m7GTP, we demonstrate that disruption of this pathway attenuates both internal m7G modification and SARS-CoV-2 replication. These findings position NSP14 as a critical effector in coronavirus-driven epitranscriptomic reprogramming and highlight internal m7G modification as a novel regulatory axis in host-virus interactions. Our work expands the understanding of how coronaviruses subvert host RNA metabolism and identifies NSP14 as a promising therapeutic target.

Lecture

mTOR signaling and hexosamine biosynthesis: supply and demand dynamics during early T cell development

Estela Jacinto, PhD
Rutgers University

Cell fate decisions are influenced by various factors, including nutrient availability. However, the mechanisms through which cells integrate these inputs to generate specific outputs, such as differentiation or proliferation, remain poorly understood. In early T cell development, the generation of a diverse repertoire of T cell receptors (TCRs) is crucial for establishing a robust immune system capable of recognizing a wide range of pathogens while remaining tolerant to self-peptides. The synthesis of this diverse repertoire relies on abundant metabolites, such as hexosamines, which are essential for the proper synthesis, glycosylation, and folding of the TCR. In this study, we explore how mTOR signaling regulates the supply of these metabolites in response to nutrient demands during thymocyte development. We investigate how T cell ontogeny and TCR diversity are influenced by de novo hexosamine biosynthesis and examine the effects of dietary supplementation of key metabolites on these processes. By enhancing our understanding of how nutrient supply is regulated in accordance with cellular demand, we aim to develop dietary strategies that could potentially improve immune system function.

Lecture

Uncovering a pandemic's cause, and gene delivery mechanisms, by virus nanoimaging

Jason Kaelber, PhD
Rutgers University

Faced with a nationwide agricultural epidemic, we used cryo-electron microscopy to discover the etiological agent, a novel parvovirus. Tests of Koch's postulates, characterization of the agent, and countermeasures will be described. Extending cryo-EM to visualizing viral events inside the cell, we obtained insights into the trafficking of adeno-associated virus, a human-infecting virus often used in gene therapy.

Lecture

Evaluating the barriers to cross-species virus infections

Ryan Langlois, PhD
University of Minnesota

The global virome is incredibly diverse and emerging viruses threaten global health. The risk of virus spillover from one host into another is increasing due climate change. Currently it is difficult to determine which viruses among the many thousands have the potential to infect humans and drive novel pandemics. Viruses face many barriers when encountering a new species. They must use host factors to

enter and replicate and evade the antiviral immune system. How viruses overcome these barriers could help to uncover new virus-host biology and help inform pandemic risk prediction. To address this, we have developed a novel model system to evaluate cross-species infection potential. We take an unbiased approach and measure replication of a library of viruses in a "fibroblast zoo", which represent the diversity of Mammalia. Using this resource we can evaluate entry and innate immune barriers to cross species infections.

Lecture

Mitochondrial Dynamics as a Nexus for Alphavirus Replication Strategies

Maudry Laurent-Rolle, MD, PhD
Yale University School of Medicine

The innate immune response is the body's first line of defense against viral infections. Mitochondria are dynamic organelles continuously undergo fusion/elongation and fission, regulating various aspects of mitochondrial biology, including innate immunity. Enhanced mitochondrial fusion/elongation boosts innate immune responses by increasing interactions between mitochondria and the endoplasmic reticulum (ER), leading to increased interferon production and inhibition of viral replication. Orthoflaviviruses like Zika, dengue and yellow fever virus manipulate mitochondrial dynamics and bioenergetics to enhance their replication. However, less is known about the impact of alphaviruses on mitochondrial dynamics and bioenergetics. In our study, we conducted a comprehensive investigation into whether both Old World and New World alphaviruses disrupt mitochondrial dynamics, and we observed variations not only between these Old World and New world alphaviruses but also within the New World alphaviruses themselves. Understanding these interactions is crucial for deciphering alphavirus pathogenicity and advancing therapeutic development to mitigate their impact on mitochondria.

Lecture

Maternal-Offspring Immune Partnership

Ai Ing Lim, PhD
Princeton University

Mothers and offspring are evolutionary partners. From gestation to lactation, mothers undergo profound immunological transformations that are essential to tolerate and nurture semi-allogeneic offspring, while simultaneously responding to environmental challenges. During this window, maternal exposure to environmental factors—from pathogens to pollutants—can markedly influence offspring immune system development, prenatally through the placenta and postnatally through breastfeeding. These influences are not transient but can permanently shape offspring immunity and alter lifelong susceptibility to infection and inflammation. In my talk, I will discuss how maternal barrier tissue immunity adapts throughout reproduction, and how maternal helminth infection enhances antiviral immunity in offspring.

Lecture

Immune Evasion and Cell-to-Cell Transmission of SARS-CoV-2

Shan-Lu Liu, MD, PhD
The Ohio State University

We focus on virus–host interactions, with particular emphasis on host factors that modulate viral membrane fusion, entry, and release. Over the past several years, Dr. Liu's research has provided significant insights into how host restriction factors, such as IFITM, LY6E, TIM, and SERINC, impede infections by HIV, Ebola virus, Zika virus, and SARS-CoV-2, and how these viruses evolve mechanisms to evade such host defenses. His COVID-19 research has focused on the molecular and cellular mechanisms of SARS-CoV-2 entry, membrane fusion, cell-to-cell transmission, immune responses, and vaccine development. In this presentation, Dr. Liu will discuss host immune responses to COVID-19 vaccination and natural infection, as well as mechanisms of cell-to-cell transmission of HIV, Ebola virus, and SARS-CoV-2.

Lecture

The SARS-CoV-2 RNA cap methyltransferase NSP14: Exploring new targets for antiviral drug development

Cindy Meyer, PhD
The Rockefeller University

Coronavirus disease 2019 (COVID-19) is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Despite extraordinary progress in developing vaccines and first-generation small molecule therapies, there remains an urgent need for safe and effective orally bioavailable drugs that can be used in the outpatient setting. We recently developed a first-in-class non-covalent small-molecule inhibitor of the SARS-CoV-2 guanine-N7 methyltransferase NSP14. High-throughput screening identified RU-0415529, which inhibited SARS-CoV-2 NSP14 by forming a unique ternary S-

adenosylhomocysteine (SAH)-bound complex. Hit-to-lead optimization of RU-0415529 resulted in TDI-015051 with a KD of 61 pM and an EC50 of 11 nM inhibiting virus infection in a cell-based system. TDI-015051 also inhibited viral replication in primary small airway epithelial cells and in a transgenic mouse model of SARS CoV-2 infection with an efficacy comparable to the FDA-approved reversible covalent protease inhibitor nirmatrelvir6. The inhibition of viral cap methyltransferases as an antiviral strategy is also adaptable to other pandemic viruses.

Lecture

***Immunoresponsive gene 1* supports collagen remodeling following sterile injury**

Veronika Miskolci, PhD
Rutgers University

Immunoresponsive gene 1 (*Irg1*), or *ACOD1*, encodes aconitate decarboxylase 1, a mitochondrial enzyme that produces itaconate primarily in myeloid cells. Itaconate is an immunomodulatory

metabolite, critical in macrophage immunometabolism. While their roles and mechanisms of action are extensively studied in host-pathogen interactions, they are less understood in sterile inflammation. Here, we study the role of *Irg1* in sterile inflammation using a larval zebrafish model of inflammation and tissue repair. Using the Tg(*irg1:EGFP*) transgenic line, we observed that *irg1:EGFP* expression in macrophages increases shortly after they arrive at the wound, peaking by 24 hours and resolving over time following caudal fin transection. We found that *Irg1:GFP* expression is regulated by myeloid-derived growth factor (MYDGF) and is independent of MyD88 signaling at the wound. Our previous report showed that MYDGF limits sterile inflammation during tissue damage, but not infection. *Irg1* depletion does not affect macrophage recruitment, but results in neutrophil accumulation at the wound. *Irg1* depletion impairs wound healing, evidenced by diminished collagen fiber formation, and fewer vimentin-expressing cells and collagen-based epithelial projections at the wound. Live imaging shows that macrophages frequently hover at the epithelial projections in control, but this interaction is markedly altered in Irg1-depleted animals. Our findings reveal a potential role for *Irg1* in regulating collagen remodeling by macrophages in the wound microenvironment. The function of macrophages in the formation of epithelial projections and the dependence on itaconate remain to be examined.

Lecture

CSF2 induced metabolic changes underlying dendritic cell development from monocytes.

Edward Pearce
Johns Hopkins University

Our laboratory has a focus on understanding how environmental signals alter cellular metabolism to influence cellular activation and differentiation. In this context, we have been exploring the effects of CSF1 and CSF2 on monocytes. Despite signaling through distinct receptors, linked to distinct signaling pathways, both of these cytokines drive the differentiation of monocytes into macrophages, but CSF2 can additionally drive differentiation into cells that exhibit dendritic cell (DC)-like characteristics (Mo-DCs). I will discuss our finding that a metabolic response initiated by CSF2, but not CSF1, favors Mo-DC differentiation.

Lecture

Regulation of Immunity and Virus Replication by Host Posttranslational Modifications, and Novel Therapeutic Opportunities

Ricardo Rajsbaum, PhD
Rutgers University

The host antiviral innate immune response involves activation of multiple signaling pathways that result in the production of type I interferons (IFN-I) and inflammatory cytokines, which together control virus infections. However, excessive inflammation and cytokine production can promote exacerbated disease. To achieve the right balance, these signaling pathways are regulated by posttranslational modifications (PTMs), including phosphorylation and ubiquitination. These PTMs can regulate antiviral immunity to protect against infection. To counter these host defenses, viruses have developed mechanisms to hijack

PTMs to enhance their own replication. Our lab studies the interactions that occur between the host innate immune response, posttranslational modifications and different highly pathogenic viruses including Ebola, SARS-CoV-2 and Influenza. We will discuss the identification of chemical compounds targeting PTMs that can help study molecular mechanisms and at the same time provide new opportunities for the development of antiviral and anti-inflammatory approaches.

Lecture

Neutrophils and interferons meet at unexpected places

Amariliz Rivera, PhD
Rutgers University

Neutrophils are well-known early effectors of pathogen eradication and are essential for defense against invasive fungal infections. Neutrophils are also increasingly recognized as versatile immune regulators capable of surprising diversity. In previous studies, we discovered that the antiviral cytokines type I and III interferons (IFNs) are essential regulators of antifungal immunity. Using gain and loss of function approaches we determined that type III IFNs (also known as IFN-l) are direct regulators of antifungal neutrophils for protection against *Aspergillus fumigatus* (Af). Whether infection with Af (a clinically relevant mold pathogen) activates similar cellular sources of protective IFN-l as well-known responders to viral infection is currently unclear. In this study, we set out to identify the relevant biological sources of IFN-l during fungal infection. We discovered that antifungal neutrophils are the primary source of IFN-l during pulmonary Af infection. Conditional removal of the entire IFN-l locus on neutrophils (IFN-l2/3ΔPMN) significantly reduced global IFN-l levels in the infected lung and rendered mice susceptible to invasive aspergillosis (IA). Neutrophils from IFN-l2/3ΔPMN mice showed defective reactive oxygen species (ROS) formation and reduced fungal killing. We determined that neutrophil-derived IFN-l production relies on a feedforward loop activated by type I IFN. Accordingly, conditional removal of IFNAR on neutrophils results in significantly reduced expression of IFN-l and impaired antifungal immunity against Af. Expression of neutrophil-derived IFN-l is further amplified by engagement of the type III IFN receptor. Hence, conditional removal of IFNLR1 on neutrophils leads to diminished expression of IFN-l in the lung of Af-infected mice. Altogether, our studies identify neutrophils as a novel, indispensable source of protective IFN-l during fungal infection.

Lecture

Uncovering bacterial and host interactions driving heterogeneity in M. tuberculosis transmission and infection outcome.

Padmini Salgame, PhD
Rutgers University

Despite advances in the development of new diagnostics, vaccine candidates and drugs, tuberculosis (TB) continues to endanger global health. A major knowledge gap is an incomplete understanding of the transmission dynamics of *Mycobacterium tuberculosis* (Mtb) and the subsequent infection outcome in individuals exposed to the pathogen. A household contact (HHC) study conducted by our group in Brazil found heterogeneity of Mtb transmission within households. Households (HH) were categorized

into High (HT), and Low (LT) transmission groups based on the proportion of HHC with a positive Interferon-Gamma Release Assay (IGRA) and tuberculin test. Mtb strains from index cases of the HT and LT households were designated Mtb-HT and Mtb-LT, respectively. The presentation will encapsulate findings from our studies in human cohorts and experimental animal models that provide novel insights into the distinct immunological mechanisms induced in Mtb-HT and Mtb-LT infected hosts that could influence *M. tuberculosis* transmission and infection outcome.

Lecture

Metabolic memory, epigenetics, and increased susceptibility to sepsis in diabetic conditions.

Henrique Serezani**,** PhD
Vanderbilt Institute for Infection, Immunology and Inflammation

Despite glucose monitoring and insulin therapy, many individuals with diabetes remain hyperglycemic for long periods, elevating their risk of comorbidities, including a higher susceptibility to infections. We have demonstrated that diabetic and obese mice are more susceptible to *S. aureus* infection, as indicated by increased mortality, bacterial loads and kidney damage. However, the mechanisms underlying increased mortality in septic diabetic conditions remain to be fully understood. These events may be driven by the epigenetic phenomenon of metabolic memory, wherein early and transient hyperglycemic events induce epigenetic modifications that predispose target organs to produce abundant inflammatory mediators, leading to diabetes-associated morbidities. We hypothesize that hyperglycemia1 primes histone acetyltransferase (HAT) activation to promote sustained and amplified cytokine production, resulting in an intense but ineffective inflammatory response to systemic infection, leading to severe MODS and increased mortality rates. Our data indicate that insulin, but not Phlorizin (a SGLT2 inhibitor that reduces glucose levels), improves the survival of septic diabetic mice. Next, we assessed whether changes in the sustained expression of inflammatory genes in diabetes might relate to enhanced HAT activity. Our data show that macrophages from diabetic mice show increased p300 HAT activation and H3K27 acetylation. HAT activation is regulated by the localization and availability of its substrate, Acetyl-CoA. Our data show increased Acetyl-CoA production in areas near the abscesses of diabetic mice. High glucose leads to activation of ATP citrate lyase (ACLY) phosphorylation and macrophage-mediated Acetyl-CoA production during MRSA infection. ACLY inhibition improves the survival of diabetic mice, which correlates with reduced systemic production of inflammatory mediators and kidney damage markers. Understanding how altered metabolism and epigenetic modifications in the context of inflammation and sepsis holds tremendous potential for pioneering therapeutics. These advancements could specifically target the reduction of chronic low-grade inflammation in diabetes, thereby significantly enhancing the outcomes of systemic infections linked to this debilitating disease.

Lecture

Immune control of the metabolic stress responses to infection.

George Yap, PhD
Rutgers University

Sickness associated anorexia is a conserved feature of disease. Here, I will discuss our recent discoveries regarding how immune cytokines regulate 1) the mediators that depress food intake and body weight and 2) the adaptive metabolic responses activated the liver to compensate for decrease nutrient availability.

Lecture

Contrasting roles of MERS-CoV and SARS-CoV-2 internal proteins in pathogenesis

Roy Wong, PhD
Rutgers University

Betacoronaviruses encode an internal (I) gene via an alternative reading frame within the nucleocapsid gene, called ORF8b for Middle-East respiratory syndrome coronavirus (MERS-CoV) and ORF9b for severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2. Previous reports suggested that proteins 8b and 9b are involved in evading multiple innate immune signaling pathways. However, their roles in mediating pathogenesis in infected animals have not been determined. In this study, we abrogated the expression of protein 8b in MERS-CoV and protein 9b in SARS-CoV-2. Using mouse models of MERS-CoV and SARS-CoV-2 infection, we found that MERS-CoV lacking protein 8b expression was more virulent, while SARS-CoV-2 lacking protein 9b expression was attenuated compared with the respective wild-type viruses. Upon further analysis, we detected increased levels of type I interferon and enhanced infiltration of immune cells to the lungs of mice infected with MERS-CoV lacking protein 8b expression. These data suggest that the I protein of MERS-CoV plays a role in limiting pathogenesis while that of SARS-CoV-2 enhances disease severity.

Lecture

Diet-mediated immunometabolic regulation promotes *K. pneumoniae* airway clearance

Tania Wong, PhD
Rutgers University

Antimicrobial-resistant and susceptible *Klebsiella pneumoniae* (Kp) are major causes of pneumonia and mortality in healthcare settings. One emerging immune evasion strategy of Kp involves its manipulation of the host metabolism, particularly through the induction of mitochondrial oxidative phosphorylation (OXPHOS). The airway metabolic milieu promotes the accumulation of immunosuppressive myeloid cells that fail to clear the bacteria. We hypothesized that a ketogenic diet would limit anti-inflammatory cells and enhance bacterial clearance through diet-induced ketones, which improve immune cell bioenergetics and function.

Using a mouse model of pneumonia, we compared the bacterial burden in mice fed either a ketogenic or control diet. Mice on the ketogenic diet had elevated ketone levels in both the blood and airway, under baseline conditions and during infection. Notably, these mice exhibited reduced pulmonary bacterial loads and improved survival. We observed significantly higher numbers of neutrophils, monocytes, macrophages, and T cells in their lungs. Neutrophils, monocytes and macrophages from the infected mice on the ketogenic diet showed reduced expression of the exhaustion marker PDL-1 compared to those on the control diet. They also had decreased levels of acetyl-CoA carboxylase (ACC), which typically diverts ketone-derived acetyl-CoA toward lipid synthesis rather than ATP production. Instead, these cells exhibited enhanced AMPK signaling and improved effector function. Our data highlight the role of ketones in enhancing the immune response to Kp by supporting bioenergetics, suggesting that regulating the host metabolism may help clear persistent infections.

Notes

The role of the deubiquitinase USP11 during Ebola Virus Infection

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The Ebola virus (EBOV) causes a severe and fatal disease by disrupting the balance of the immune system, leading to heightened inflammation. To explore the mechanisms of immune dysregulation, we compared changes in protein abundance and posttranslational modifications (PTMs) in primary cells from healthy donors upon EBOV infection. We focused on changes in expression of deubiquitinases, which many are known to regulate inflammatory pathways. To gain information on potential dysregulated immune signaling pathways during infection, we used a WT EBOV and a recombinant VP35 mutant that lacks the ability to antagonize IFN-I (EBOV-VP35mt). Mass spectrometry (MS) analysis from EBOV infected human monocytes, CD4+ and CD8+ T cells revealed downregulation of Ubiquitin-specific protease 11 (USP11) expression upon infection with WT EBOV in monocytes. USP11 is a host deubiquitinase that modulates immune signaling and viral replication during Ebola virus (EBOV) infection. Here, we show that USP11 exerts a proviral role by fine-tuning innate immune responses and directly regulating the viral polymerase cofactor VP35. EBOV infection in USP11 knockout cells resulted in increased expression of interferon-β (IFN-β) and the proinflammatory chemokine CXCL1, suggesting that USP11 regulates antiviral cytokine production. Co-immunoprecipitation studies revealed that USP11 interacts with the host kinase IKKε, implicating a potential mechanism of immune modulation. Notably, USP11 knockdown led to increased ubiquitination of EBOV VP35, and mass spectrometry analysis identified two USP11-sensitive lysine residues, K216 and K222. Mutation of K216 to arginine (K216R) significantly enhanced viral minigenome activity, indicating that ubiquitination at this site regulates VP35 function. Despite only a modest reduction in viral titers, USP11 knockout cells infected with wild-type EBOV exhibited excessive cytokine expression at 48 hours post-infection, as revealed by RNA-sequencing, highlighting USP11’s critical role in maintaining immune balance.

Title: Immunodominant structural proteins Gc and N drive T cell-mediated protection against La Crosse virus

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Abstract

La Crosse virus (LACV), a negative-sense RNA bunyavirus, is a major cause of pediatric encephalitis in the United States, disproportionately affecting children under the age of 16 years. Although LACV can lead to severe morbidity and mortality in this vulnerable pediatric population, no vaccines or specific antiviral therapies are currently available. Murine models recapitulate the age-dependent susceptibility observed in humans, whereby weanling mice (3 weeks old) succumb to LACV-induced neurological disease and adult mice (≥8 weeks old) exhibit resistance. In this study, we systematically characterized the T cell-mediated responses that underlie this differential susceptibility. We show that adult mice mount robust, polyfunctional LACV-specific $CD4^+$ and $CD8^+$ T cell responses against structural and non-structural proteins as early as 6 days post-infection (dpi), with sustained production of interferon-gamma (IFN-γ),

granzyme B, interleukin-2 (IL-2), and tumor necrosis factor alpha (TNF-α). We further show that these T cells expand significantly in adult mice and display in vivo cytotoxicity against targeted cells pulsed with immunogenic antigens derived from the envelope Gc and nucleocapsid (N) proteins. In contrast, weanling mice exhibit significantly weaker T cell responses, as evidenced by reduced expansion of both $CD4^+$ and $CD8^+$ T cells and diminished cytokine production, correlating with 100% mortality by 7 dpi. Importantly, immunization studies with LFn-LACV Gc and N proteins significantly improve in vivo cytotoxicity and survival in weanlings, highlighting their potential as vaccine candidates. Collectively, our findings define key cellular correlates of protection against LACV and establish a framework for the development of pediatric vaccines designed to mitigate the public health burden of LACV-induced encephalitis.

Astrocytic p53 Orchestrates Flavivirus Neurotropism via Metabolic and Glycosylation Reprogramming

Neurotropic flaviviruses, including Zika virus and West Nile virus, are growing threats to global public health. Recent work has underscored roles for regulation of cellular metabolism in driving protective host responses to flavivirus infection. While the canonical tumor suppressor protein p53 is recognized for its role in maintaining genomic stability and regulating cellular metabolism in cancer, its potential role in shaping metabolism during viral infection remains largely unexplored. We show that p53 promotes flavivirus replication and neuropathogenesis, specifically by modulating the metabolic responses of astrocytes. Using both pharmacologic and genetic approaches, we show that abrogation of p53 signaling in astrocytes during flavivirus infection drives a metabolic shift into aerobic glycolysis, reminiscent of the Warburg effect observed in cancer cells. Strikingly, the absence of p53 in astrocytes dramatically reduces viral replication and improves clinical outcomes in multiple animal models of infection. This enhanced virologic control is driven by glycolysis-dependent O-glycosylation of key antiviral glycoproteins that suppress flavivirus replication in the setting of p53 ablation and unrestrained astrocytic glycolysis. Together, our findings identify unexpected mechanisms of immunometabolic regulation and host-pathogen interactions in astrocytes.

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Helminth infection favors persistent and proliferating alternatively activated neutrophils in the lung

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Neutrophils are granulocytic myeloid cells that have critical effector functions during infectious disease but are typically thought to be short-lived, terminally differentiated phagocytic cells. We directly compared lung neutrophil activation following infection with different groups of pathogens including bacteria (*Staphylococcus aureus*), fungi (*Aspergillus fumigatus*), and helminths (*Nippostrongylus brasiliensis*) at 2 days after infection. Our results showed considerable heterogeneity depending on the type of infectious agent. Bulk RNAseq, EdU incorporation, and flow cytometric staining for necrosis/apoptosis identified distinct activation states with rapid cell death predominant in neutrophils after *S. aureus* and *A. fumigatus* infection, while neutrophils from helminth infected mice exhibited increased cell cycling and expression of signaling pathways associated with proliferation, type 2 responses, and wound healing. Spectral flow cytometry, intravascular staining, and scRNAseq revealed a distinct c-kit+ proliferating subset of lung neutrophils that expanded shortly after helminth infection and was released from the endothelial niche to co-localize with invading parasites in the airways. These findings challenge current models of neutrophil function during infectious disease, suggesting that neutrophils uniquely assume a persistent and proliferative phenotype that may contribute to tissue repair and resistance in the context of the type 2 pulmonary inflammatory response.

† These authors contributed equally to this work.

The Host Mannose Receptor Type C1 (MRC1) Acts as an Ebola Virus Entry Factor

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Ebola virus disease, caused by Ebola virus (EBOV), a member of the *Filoviridae* family, is life-threatening due to immune dysregulation. We are working on a collaborative, multiplatform project to comprehend the mechanism of immune dysregulation during EBOV infection at the DNA-to-RNA-to-protein level. We compared the landscape of protein abundance in the human proteome in primary, healthy donor cells (monocytes, CD4+ and CD8+ T cells) infected with wild-type (WT) EBOV and a recombinant VP35 mutant that lacks IFN-I antagonism (EBOV/VP35m). RNA-seq and mass spectrometry analysis from these cells revealed the downregulation of a gene cluster involved in virus entry and receptor activity during infection. Specifically, we found downregulation of the Mannose Receptor type C1 (MRC1) gene, a type-I transmembrane protein with reported roles in pattern recognition receptor and virus attachment. To elucidate the role of MRC1 in EBOV lifecycle, we used both an EBOV-reporter Virus-like particle (TrVLP) system and the Zaire EBOV. Knockdown of MRC1 in primary donor monocytes or MRC1 CRISPR knockout THP1-differentiated cells displayed reduced VLP attachment and entry.

Additionally, these cells showed reduced virus titre upon EBOV infection. Interestingly, the induction of inflammatory cytokines was reduced in MRC1-knockdown cells upon treatment with these VLPs or EBOV. This suggests that MRC1 might recognize EBOV on the cell surface and trigger immune signaling. This is further supported by the observation that FACS-sorted $MRC1^{+}$ mouse bone marrow-derived macrophages (BMDM) have an increased VLP uptake compared to the $MRC1^{-}$ BMDMs. The Co-immunoprecipitation (Co-IP) assays showed specific binding of EBOV GP with MRC1. Using structural modeling and co-IP we found that among the eight carbohydrate recognition domains (CRDs) of MRC1, CRD-4 and CRD-8 are involved in binding with GP. To our surprise, the TrVLP-treatment in primary donor monocytes and *ex-vivo* study with mouse cells revealed that MRC1 downregulation during EBOV infection appears to be cell-type specific. This tissue tropism is also supported by our Non-Human Primate (NHP) study using EBOV. Since MRC1 is also a marker of type-2 macrophages, we propose that MRC1 is an attachment factor for EBOV, specifically increasing EBOV entry in type-2 innate immune myeloid cells, which also triggers inflammatory response, and downregulation of MRC1 may be a cellular mechanism to dampen inflammation and virus entry.

Investigating the contributions of hematopoietic stem and progenitor cells to antihelminth immunity and host protection

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Protective responses to helminth parasites are dependent on type 2 cytokine-mediated inflammation that is required for worm expulsion and the healing of damaged tissues. These events are critically supported by various cell populations including mast cells. Our previous studies have identified a population of hematopoietic progenitor cells (HPCs) that possess mast cell potential and are defined by their expression of the metabolic enzyme carbonic anhydrase (Car)1. Car1-expressig progenitors exit the bone marrow and traffic to inflamed tissues following a *Trichinella spiralis* infection. Despite these advances, the relative contributions of Car1-expressing progenitors to the infection-induced increases in mast cells and their contribution to antihelminth immunity remain unknown. To address this, we have developed a novel Car1-ERCre mouse to perform important fate-mapping and lineage deletion studies. Here we demonstrate that our novel mouse model can be used to selectively label *Trichinella*-induced mast cells with a history of Car1-expression. Furthermore, deleting the Car1-expressing progenitors not only significantly reduced mast cell development, but also resulted in decreased protective immunity in the context of a *T. spiralis* infection. Collectively, our data suggests that Car1-expressing progenitors critically support infection-induced mast cell responses and represent a therapeutic target for the treatment of mast cell-mediated inflammation.

Keywords: Type 2 immunity, Mast cells, Helminths, Hematopoietic progenitor cells, Inflammation

Exploring the effect of diet on *Bifidobacterium* colonization in the gut using a gnotobiotic mouse model

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Bifidobacterium is a probiotic commensal microbe exhibiting various health-promoting effects in humans. Since efforts to reintroduce *Bifidobacterium* in adults have failed for unclear reasons, uncovering the factors impacting the intestinal persistence of *Bifidobacterium* is necessary to develop new strategies to restore this beneficial microbe and treat inflammatory diseases and cancer. We found that both the host adaptive immune system and dietary iron did not impact the colonization levels of *Bifidobacterium breve* in mono-colonized mice. Instead, we observed a significant decrease in *B. breve* colonization upon diet switch from a standard grain-based lab diet to a purified AIN-93G diet containing non-fermentable fiber. This rapid diet-dependent decrease was observed in mice mono-colonized by *B. breve* and in mice co-colonized by *B. breve* along with a small bacterial consortium. Surprisingly, supplementing the purified diet with inulin and pectin fiber, which are reported to enhance *Bifidobacteria* abundance in human studies, could not rescue this decrease. Bacterial RNA-seq analysis showed that the expression of stress response-related genes in *B. breve* was upregulated upon diet switch, while that of genes associated with carbohydrate metabolism, particularly raffinose metabolism, was downregulated. Notably, supplementing raffinose in drinking water dramatically rescued the diet-dependent decrease of *B. breve* in mono-colonized and consortium-colonized mice. Moreover, introducing *B. breve* to the consortium-colonized mice significantly increased epithelial barrier gene expression in the ileum and colon upon tumor necrosis factor (TNF) injection, implying a potential protective effect of *B. breve* against TNF-induced intestinal inflammation. Metabolomic analysis further revealed that elevated *B. breve* levels upon raffinose supplementation led to higher levels of indole-3-lactic acid, a health-beneficial bacterial metabolite, in the cecum of consortium-colonized mice. Altogether, our results show that specific dietary fibers significantly modulate the intestinal persistence, metabolism, and physiology of *Bifidobacterium*, which is potentially linked to its altered probiotic functions.

Metabolomic signatures of the steroid biosynthesis driving sex differences in clinical asthma subtypes

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Abstract

Background: Asthma is a heterogeneous disease with distinct prevalence between sexes. However, the role of steroids and medication use in asthma between sexes over puberty remains unknown. Metabolites associated with the general diagnosis of asthma have been instrumental in identifying common dysregulation among all individuals with asthma. However, understanding phenotype-specific metabolites and their differences by sex would additionally provide insight into distinct pathophysiology that more accurately classifies specific asthma subtypes with potential for gender-specific personalized asthma treatments.

Methods: Targeted whole blood metabolomic profiles were generated from Precion Inc, NC. Sixteen metabolites from the steroid biosynthesis pathway were quantified in 958 asthmatic children from the Childhood Asthma Management Program over the ages of 9-16 years (60.8% males). Linear and generalized mixed models were used to investigate the effect of treatment/Inhaled corticosteroid (ICS) use (exposure) on the metabolite concentrations (outcome) adjusting for age, sex, race, and body-mass-index. Further, a stratified analysis by sex and ICS was performed to evaluate differences by treatment between sexes. Metabolite associations with other clinical and inflammatory phenotypes of asthma were also evaluated including lung function, total eosinophils as a diagnostic cutoff of total EOS $\geq$ 300/μL and total neutrophils as quartiles. Significance was determined by a p-value < 0.05.

Results: Testosterone, dehydroepiandrosterone sulfate, and alpha-hydroxyprogesterone levels were significantly reduced with ICS use across both males and females. Upon stratifying by sex, striking differences were observed. In females, ICS use significantly reduced multiple androgen sub-pathway metabolites, including dehydroepiandrosterone sulfate and aldosterone. In males, testosterone levels were significantly decreased with ICS exposure. Age-related pre- and post-puberty window analyses further revealed that metabolites from the androgen and progestogen sub-pathways—particularly testosterone and progesterone—exhibited the most pronounced ICS-

related reductions in females. In contrast, testosterone remained the primary metabolite significantly affected by ICS use in males. Moreover, all sixteen measured steroid metabolites showed strong associations with lung function and inflammatory phenotypes, suggesting that steroid sub-pathways — including glucocorticoids, mineralocorticoids, progestogens, and androgens — play a critical role in regulating inflammatory responses in both sexes. Importantly, the differential patterns of steroid metabolite suppression between males and females suggest that ICS treatment may unmask or exacerbate underlying sex-specific vulnerabilities in steroid metabolism. Females exhibited broader suppression across multiple steroid pathways, while males showed more targeted effects, predominantly on testosterone. These findings imply that steroid biosynthesis and endocrine compensation mechanisms may differ between sexes, influencing hormonal balance and susceptibility to lung dysfunction and inflammation. Consequently, prolonged ICS use could pose different endocrine and respiratory risks for males and females, potentially contributing to sex-specific trajectories in asthma severity, lung injury, or corticosteroid-induced adrenal suppression.

Conclusions: We show ICS-associated metabolite differences between males and females over puberty and suggest that therapeutic strategies targeting these metabolite-biomarkers for adrenal insufficiency should be different between sexes.

Evan DaPrano – BHI 2024 Abstract

Title of Abstract: Asymptomatic adult Zika virus infection incurs long term neurological consequences

Authors: Evan M. DaPrano, Irving Estevez, Marissa Lindman, Benjamin D. Buckley, Cara Nasello, Joshua S. Thackray, Colm Atkins, Max A. Tischfield, and Brian P. Daniels

PI Name: Brian Daniels

Abstract: Recent work has underscored the potential for chronic neurological consequences following viral infections. Although mild and asymptomatic cases make up the vast majority of human viral infections, virological studies to date have overwhelmingly focused on models of severe infection and encephalitis. Zika virus is an emerging flavivirus of global concern that is most known for its connection with congenital sequalae following infection in utero. However, despite possessing tropism for neurons, astrocytes, and neural progenitors, infection in healthy adults is asymptomatic in approximately 80% of cases. We have developed an immunocompetent mouse model of systemic ZIKV infection in which infected animals do not display any clinical illness or differences in weight gain, despite ZIKV being detectable in the brain. In this asymptomatic model, we demonstrate leukocyte recruitment into the brain and the establishment of brain tissue resident memory cells (TRMs) up to 32 weeks post infection. Transcriptomic and metabolomic approaches demonstrate dysfunction in pathways related to inflammation, neurodegeneration, and senescence in the postinfectious brain, while computationally driven behavioral approaches illustrate perturbed movement patterns in freely moving mice at 32 weeks post infection. This project will define the neurologic consequences of asymptomatic ZIKV infection while following experiments will describe roles for long-lived brain resident T cells in promoting neurologic sequelae in the postinfectious brain.

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Fibrinogen activates microglia through CD11b binding and regulates hypothermia in hyperinflammatory experimental cerebral malaria.

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3rd-year Infection, Immunity, Inflammation PhD

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Abstract

Cerebral malaria (CM), caused by *Plasmodium* parasites, causes the deaths of over 600,000 people each year. Brain pathology includes vascular congestion, hypercoagulation, and neuroinflammation. *P. chabaudi* infection of IL-10 KO animals leads to systemic inflammatory cytokine increase and mortality. As fibrin(ogen) (Fib) can directly activate the brain resident macrophages, microglia via CD11b, interactions between fluorescent fibrinogen (Fib-A647) and microglia were investigated in $CX3CR1^{GFP}$-$CCR2^{RFP}$ IL-10 KO reporter mice. *P. chabaudi*-infected reporter mice showed activated $CX3CR1^{GFP+}$ microglial morphology by confocal microscopy, including internalized Fib-A647. Both $CCR2^{RFP+}$ and $CX3CR1^{GFP+}$ cells actively interacted with brain vasculature at day seven post-infection. $CCR2^{+}$ cells were found primarily within the vasculature, including adherent T cells and inflammatory monocytes. These leukocytes were not adherent to the vasculature by integrins, as they were not dislodged by neutralizing ICAM1/2 or VCAM. Leukocytes remained in the vasculature after treatment with an anticoagulant. Within the brain parenchyma, activated microglia are also recruited to the vasculature. The chemokine CCL5 was previously identified within clotted vessels near microglia. Here, intranasal administration of a CCL5 inhibitor yielded a decrease in microglia recruitment to the vasculature. Though anticoagulants ameliorate disease, no change in disease severity was observed upon infection of mice with fibrinogen mutated to reduce clotting ($Fib^{AEK,}$ $Fiba^{KO}$) with *P. berghei* ANKA (*Pb*A), even after depletion of platelets. The interaction of Fibrin(ogen) with microglia via CD11b binding was blocked with an intranasal inhibitory peptide (Fibg377-395), or in fibrinogen gamma mutant animals (390–396A). Inhibition of Fib-CD11b interaction significantly reduced microglial activation during infection, but increased disease severity, particularly hypothermia. These findings suggest that the interaction of fibrinogen with microglia contributes to disease tolerance.

Keywords: Experimental cerebral malaria, microglia, coagulation, neuroinflammation.

Acknowledgments

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MBGSO Grad Student Symposium Abstract
Title: Identifying the Interactome of the key regulator, H2-O, in the MHC-II Antigen Presentation Pathway

Ryan Fink

Extracellular pathogens are recognized by specialized antigen-presenting cells (APCs), which process and present peptides via major histocompatibility complex II (MHC-II) to CD4 T cells, thereby initiating antibody-mediated immunity. Peptide loading of MHC-II is catalyzed by H2-M and its negative regulator H2-O. However, recent evidence reveals that the understanding of H2-O function is incomplete. Specifically, I/LnJ mice clear mouse retroviruses while C57Bl/6J mice are susceptible. Gene mapping studies revealed that four single nucleotide polymorphisms (SNPs) in the *H2-Ob* gene of I/LnJ mice result in four amino acid substitutions that mediate the retroviral response via increased production of neutralizing antibodies. Notably, knock-in of three of the I/LnJ Ob amino acid substitutions into C57Bl/6J mice were sufficient to induce neutralizing antibodies and viral resistance. Critically, the knock-in Ob protein maintains wild-type expression levels, cellular localization, and maintains binding with H2-M yet fails to inhibit H2-M. These observations suggest that the 3 amino acid substitutions may facilitate novel protein interactions with H2-O that modulate the MHC-II pathway. To elucidate these potential interactions, two complementary approaches were used: immunoprecipitation coupled with mass spectrometry and a biotin ligase proximity labeling system. IP-mass-spec identified several novel binding partners, including the v-ATPase, which was confirmed by western blot. The proximity labeling system is expected to provide additional evidence of H2-O protein interactions, potentially revealing transient interactions not captured by traditional IP-mass-spec methods. Ultimately, a comprehensive understanding of this pathway will provide critical insights into how inter-individual genetic polymorphisms in the antigen presentation pathway modulate viral pathogenesis and susceptibility.

Title: Differentiation pathway of Tbet+CD11c+ B cells influences their functional properties during acute viral infections

Rebecca L. Francis, Jason S. Weinstein

Abstract Proper B cell development into antibody-secreting cells (ASCs) is vital for protection against acute infectious agents. B cell differentiation into ASCs occurs through two main pathways: germinal center (GC) and extrafollicular (EF). In GCs, activated B cells go through multiple rounds of proliferation, somatic hypermutation, and selection from T follicular helper CD4+ T cells (Tfh). These interactions promote B cell survival, affinity maturation, and differentiation into memory B cells (MBCs) or plasma cells (PCs). MBCs quickly respond during reinfection by undergoing further differentiation into ASCs or form a new GC for further processing. PCs are long-lived ASCs that produce large quantities of antibodies for months to decades following infection. In the EF response, B cells develop into low affinity memory B cells or short-lived ASCs known as plasmablasts (PBs) that secrete low affinity antibodies to help quickly clear the pathogen. The signals B cells receive to develop through these two divergent pathways and how effector cells derived from each pathway contribute to the overall response remains unclear. To gain a better understanding of B cell development and its impact on humoral responses, we investigate a newly characterized B cell subset, Tbet+ CD11c+ B cells (TBCs) that are found in various infectious and autoimmune diseases. Following acute viral infection, TBCs primarily arise from the EF pathway in the spleen, but about 10-20% develop inside GCs. In addition to splenic origins, TBCs are also found in the infected liver during LCMV infection. We **hypothesize that the origin of TBC development imprints unique characteristics that influences their subsequent effector functions, impacting their respective contributions to acute viral infection.** Here we have characterized TBCs from the splenic EF, GC and peripheral tissues during primary viral challenge. Their distinct route of differentiation influences their development kinetics, as well as their further differentiation into effector B cell populations, including, memory B cells, GC B cells, PCs and age-associated B cells. In addition, these properties impact the role of each B cell population during secondary challenge. Together, this indicates that TBCs in different locations receive distinct signals that promote their distinct functions.

Role of CD103 in Reactivation and Maintenance of Small Intestine $CD8^+$ Trm

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$CD8^+$ tissue-resident memory T cells (Trm) occupy various non-lymphoid organs, especially the barrier surfaces, and play a critical role in homeostatic maintenance of the tissue and protection from infections. In the murine small intestine, following infection with enteropathogenic *Yersinia pseudotuberculosis (*Yptb), lamina propria (LP) Trm are heterogeneous and phenotypically characterized as $CD69^+CD103^-$ and $CD69^+CD103^+$, while intraepithelial (IE) Trm are mostly $CD69^+CD103^+$. This disparity in expression of CD103 seemingly marks differences in reactivation and function, with LP $CD103^-$ Trm undergoing more robust proliferation and cytokine production *in vivo* compared to $CD103^+$ LP Trm. However, whether the interaction of CD103 with its cognate ligand E-cadherin plays a role in regulating the differential function of Trm cells or is merely a marker of this less functional population, remains unclear. The impact of CD103 on $CD103^+$ Trm function was examined *in vitro* using anti-CD3 and E-cadherin stimulation and revealed that CD103 ligation boosted T cell receptor signaling compared to anti-CD3 stimulation alone. These findings suggest CD103-dependent mechanisms enhance IE Trm responses during reactivation. Blocking CD103/E-cadherin interactions in IE Trm *in vivo* inhibited Trm reactivation and resulted in reduced Nur77 expression and cytokine production, indicating that during reactivation, CD103 has a positive impact on Trm function. To examine the importance of CD103 signaling in IE Trm throughout differentiation, CD103KO:wild-type mixed bone marrow chimeras were generated, and Yptb-specific IE Trm were analyzed by RNA sequencing. Transcripts associated with calcium signaling and T cell activation were significantly reduced in CD103KO IE Trm compared to wild-type Trm. These data indicate that CD103 impacts the phenotype of Trm at some stage of priming or maintenance within the tissue. To address the temporal role of CD103 in IE Trm function, a CD103 inducible KO model was generated and deletion of CD103 at the maintenance phase modestly reduced the number of IE Trm. Further studies with this novel CD103iKO model will help distinguish the processes of Trm differentiation and seeding within the small intestine, as well as Trm reactivation and the role CD103 plays in these conditions in diseases beyond infection.

Title:

Distinct Flavivirus Exposure Sequences Differentially Prime the Adaptive Immune Response

Abstract:

The co-circulation of flaviviruses such as dengue 1-4 (DENV-1-4), Zika (ZIKV), yellow fever (YFV), and West Nile (WNV) presents significant public health challenges due to cross-reactive immune responses that can influence disease severity. Antibody-dependent enhancement (ADE) remains a major concern, as prior immunity to one flavivirus may exacerbate subsequent infections. Using human serum samples with known exposure histories, we examined the relationship between neutralizing antibody titers and ADE potential in an Fcγ receptor-bearing human monocyte cell line. We found that prior YFV immunity did not predispose individuals to ADE, whereas prior DENV-1-4 and ZIKV infections generated highly cross-reactive antibodies capable of enhancing subsequent infections. To further investigate the impact of infection sequence on adaptive immunity, we employed a murine model to compare primary YFV-secondary DENV-1 (pYFV-sDENV-1) versus primary DENV-1-secondary YFV (pDENV-1-sYFV) infections. Both sequences led to reduced neutralizing antibody titers against the secondary infection and expansion of neutralizing antibodies against the primary infection, indicative of original antigenic sin. However, the pDENV-1-sYFV sequence resulted in a greater expansion of cross-reactive, enhancing antibodies against DENV-2 *in vitro*. Additionally, functional T-cell responses to DENV and YFV peptides were more robust in the pYFV-sDENV-1 group, suggesting an asymmetrical immune imprinting that

could influence disease outcomes. These findings highlight how flavivirus exposure history shapes adaptive immunity, with implications for vaccine strategies and risk assessment in flavivirus-endemic regions.

In Vivo Contribution of IFN-γ^{+}IL-21^{+} Th1/Tfh Hybrid Cells in Germinal Center Dynamics for Protective Immunity Response

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While Th1-like cells expressing IFN-γ^{+}IL-21^{-} control the peak of *Plasmodium* infection, Th1 cytokines can delay germinal center (GC) reaction and specific antibody responses required for parasite clearance. GC-Tfh (CXCR5^{hi}PD-1^{hi}) can promote specific antibodies and long-lived plasma cells to fully clear infection mediated by B/T interaction through IL-21. *P. chabaudi* infection has been shown to generate different cell types, including Th1-like (IFN-γ^{+}), Tfh-like (IL-21^{+}) and hybrid Th1/Tfh (IFN-γ^{+}/IL-21^{+}) cells. Therefore, we assess how these cell types influence germinal center responses and contribute to protective antibody-mediated immunity. Upon infection of triple reporter (*Il21*-kat *Il4*-GFP *Ifng-Thy1.1* KI) mice, Teff *il-21*$^{+}$ cells were enriched for GC Tfh with downregulation of CXCR6, while a significant fraction of Teff *Ifng*$^{+}$*Il21*$^{+}$ cells exhibited CXCR5^{int} CXCR6^{+} phenotype. Transfer of cytokine-expressing subsets from triple reporter and Il21$^{-/-}$ Ifng/Thy1.1 KI mice into T cell-deficient recipients, which are then infected with *P. chabaudi*, shows that *Il21*$^{+}$*Ifng*$^{-}$ cells exhibit early functionality, evidenced by the induction of specific IgG production by day 7 p.i. and confer strong protection against *P. chabaudi* infection. Both Tfh-like and hybrid Th1/Tfh cells generated more plasma cells and plasmablasts compared to Th1-like cells. Indeed, Tfh-like cell recipients generated more GC B cells, larger GCs and more parasite-specific IgG compared to other groups. While Il21^{+}Ifng^{+} hybrid T cells, also promoted GCs and specific IgG later at day 21 p.i., Th1-like *Ifng*$^{+}$ T cells from WT or Il21$^{-/-}$ mice did not, suggesting the critical role of IL-21 from both Tfh-like and hybrid Th1/Tfh in promoting protective immunity *in vivo*.

Lia Goodwin
Abstract
Yang Lab
05/01/25

Abstract for I3D Symposium & Poster Session

Title: M-CSF and GM-CSF hMDMS possess different functional activation states

Authors: Lia M. Goodwin, Jay Phansalkar, Hannah Wang, Jason H. Yang

Abstract:

Macrophages are innate immune cells that play key roles in maintaining homeostasis and preventing disease. Macrophages perform diverse functions that help clear pathogens, repair tissue, and regulate the behavior of other immune cells. These functions are regulated by the activation state of a macrophage, which is induced by extracellular cues and frequently assigned along an axis between two activation states: M1 (pro-inflammatory) vs. M2 (anti-inflammatory). However, although it is recognized that macrophages can occupy activation states beyond this axis, the functional identity of these alternative activation states remains unknown. Here we investigated the diversity in macrophage activation states using high-throughput screens for macrophage function in response to physiologically relevant signaling cues. First, we manually curated a compound library of greater than 80 molecules including cytokines, growth factors, neurotransmitters, steroids, and fatty acids. Next, we isolated monocytes from at least 4 healthy human donors per assay and differentiated them using either M-CSF or GM-CSF. We activated these primary human monocyte-derived macrophages (hMDMs) with molecules in our compound library and performed a battery of image-based functional assays for macrophage phagocytosis (pHrodo), autophagy (monodansylcadaverine), and metabolism (MitoSox and Bodipy labeling). We performed automated cell imaging and dimension reduction analyses to identify clusters of macrophage behavior, which we define as functional activation states. For each assay, we found that M-CSF and GM-CSF differentiated hMDMs responded differently to the molecules in our compound library. Moreover, we observed differences in functional behavior in hMDMs from female vs. male donors. In addition, M-CSF hMDMs exhibited less donor-donor variability across assays than GM-CSF hMDMs. Donor-donor variation was greater in the MitoSox experiments than in the Bodipy experiments, suggesting greater variability in oxidative phosphorylation vs. fatty acid utilization. Combining our measurements from across these assays, we found several functional activation states beyond the M1-M2 framework. We envision that these alternative activation states may be potentially useful for engineering next-generation immunotherapies.

Investigating the dynamics and significance of epitranscriptomics in positive-sense RNA viruses' genome

Anjali Gupta* and Chun-chieh Hsu

To date, ~140 post-transcriptional modifications (PTMs) of the canonical ribonucleotides have been discovered, yet the complexity in the genomes of RNA viruses and the elucidation of the biological significance is still in its infancy. PTMs can affect the stability of individual base pairs and establish different hydrogen-bonding patterns to redefine the higher-order structure of RNA. Moreover, PTMs influence intermolecular interactions in protein recruiting, DNA binding, and recognition of other RNA. The current study focuses on revealing novel m7G methylation sites in the SARS-CoV-2 genome. Moreover, we are exploring the role of m7G host writers like METTL1 and WDR4 in relation to the changes in PTMs and the effect of these PTMs on the phenotype and replication of the virus. This study will provide exciting insights into unexplored epigenetic modes of regulation. An essential first step toward understanding the role of RNA modifications in viral infection is to define the boundaries of the viral epitranscriptome.

Keywords: RNA viruses, Epitranscriptomics, SARS-CoV-2, m7G

Uncovering the Role of Viperin-Derived ddhC in Influenza Pathogenesis

ddhC, a small molecule produced by the antiviral protein viperin, has been detected in the serum and urine of humans, particularly during viral infections, but not during bacterial infections. During viral infections, viperin, one of the type I interferon-stimulated genes, catalyzes the conversion of CTP into ddhCTP, which plays a key role in antiviral defense. Additionally, ddhC can be imported into cells and converted into ddhCTP. It is possible that ddhCTP is the precursor of ddhC, which is secreted into the extracellular space. In vitro, ddhCTP has been shown to terminate viral RNA elongation by incorporating into nascent transcripts. Interestingly, a similar mechanism has been found in prokaryotes against viral infections, suggesting a conserved antiviral function of ddhCTP that has been shaped by evolution. Another study showed that ddhCTP can induce ribosome collisions, ultimately leading to translational shutdown. A notably high level of ddhC has been detected in the serum of influenza-infected humans. Although a previous study has shown that viperin can interfere with virus budding independently of ddhC through in vitro studies, the role of ddhC during influenza infections in both humans and mice remains largely unknown. To address this, we infected viperin knockout mice with influenza virus to examine the antiviral activity of ddhC. Surprisingly, in the absence of viperin, mice infected with influenza virus exhibited less weight loss compared to infected wild-type controls. This result indicates a protective role of viperin during influenza infection. Additionally, we generated anti-mouse ddhC antibodies to measure ddhC level in serum and urine of mice. Indeed, ddhC level were significantly elevated in wild-type mice on day 3 post-infection, but not in viperin knockout mice, confirming that viperin is required for ddhC production during influenza infection.

Zbtb20-expressing T cells as a potential regulator of cancer growth and multiple sclerosis

Vishal Karuppusamy[1], Stefan Kaluz[1], Sarah Lahire[1], Lou Osorio[1], Lisa Denzin[1,2,3] and Derek Sant'Angelo[1,2,3]

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Background: The outcome of immune responses is defined by the individual functions of T cell subsets, which are regulated by transcription factors like the PLZF-like "BTB-ZF" transcription factors. BTB-ZF transcription factor family members are crucial for immune system function as they can act as "toggle switches" between immune cell lineages (i.e., CD4 vs. CD8 T cells; innate vs. conventional T cells; B vs. GC B cells)[1]. Dr. Sant'Angelo's Lab screened for discrete expression of BTB-ZF transcription factors, specifically Zbtb20, to identify a new subset of T cells that are phenotypically and genetically distinct from others. Approximately half of these Zbtb20$^+$ T cells also expressed FoxP3, the lineage-defining transcription factor for regulatory T cells (Tregs). Importantly, they demonstrated that Zbtb20$^+$ Tregs have regulatory functions by constitutively producing IL-10. The data suggest that Zbtb20$^+$ Tregs are critical for maintaining the barrier functions of the intestine. Mice with a T cell-specific deletion of the Zbtb20 gene are highly vulnerable to experimentally induced colitis. Moreover, they have also shown that adoptive transfer of the Zbtb20$^+$ Tregs was sufficient in rescuing mice from death in DSS-induced colitis models[2]. Tregs have a unique ability to regulate and suppress the immune system, preventing it from attacking healthy tissues or creating an overly aggressive immune response by their constitutive production of the anti-inflammatory cytokine IL-10. Apart from regulating homeostasis in the intestines, IL-10 is known to modulate the function of other immune cells, such as tumor-infiltrating lymphocytes (TILs) and central system-infiltrating lymphocytes (CNS-ILs)[3,4]. Thus, we were interested in whether tumor growth and multiple sclerosis (MS) are influenced in genetically modified conditional knock-out mice (cKO mice) that cannot express the Zbtb20 transcription factor in T cells. We hypothesize that this new subset of Zbtb20$^+$ expressing T cells plays a critical role in controlling tumor progression and multiple sclerosis (MS).

Methods: To investigate the role of Zbtb20$^+$ T cells in tumor progression, we injected MC38 colon carcinoma cells subcutaneously into Zbtb20 cKO and wild-type (WT) mice. Tumor growth was monitored biweekly using digital calipers. To assess the role of Zbtb20$^+$ T cells in a model of multiple sclerosis, we induced experimental autoimmune encephalomyelitis (EAE) in both cKO and WT mice via standard immunization protocols. Mice were evaluated daily using a standardized clinical scoring system to quantify disease progression and severity. Immune profiling of TILs and CNS-ILs was performed using multiparameter flow cytometry to determine the composition, activation status, and frequency of relevant T cell subsets.

Results: In 7-month-old mice, Zbtb20 cKO animals exhibited accelerated tumor growth compared to WT controls, suggesting a tumor-suppressive role for Zbtb20 in aged mice. Conversely, in 3-month-old mice, WT animals displayed faster tumor progression, indicating a

potential age-dependent role of Zbtb20 in regulating tumor growth. In the EAE model, WT mice demonstrated markedly higher clinical scores, from onset to chronic phase, compared to Zbtb20 cKO mice, implicating Zbtb20 in the exacerbation of CNS autoimmunity. Flow cytometric analysis of CNS-ILs revealed an increased frequency of activated $CD4^+$ T cells in WT mice, including higher expression of activation and tissue-residency markers, such as $CD44^{hi}$ $CD62L^{lo}$, CD69, and CD103. These findings suggest that Zbtb20 promotes CNS inflammation by supporting the persistence and activation of pathogenic T cells.

Discussion: Our findings suggest that Zbtb20 functions in an age and context-dependent manner, promoting immune suppression in the tumor microenvironment (TME) while supporting pathogenic T cell responses in neuroinflammation. The reversal of tumor growth trends between young and older mice implies that Zbtb20 expression and function may be developmentally regulated, consistent with recent reports showing age-specific expression patterns of Zbtb20 in hematopoietic stem cells, thymocytes, and peripheral $CD8^+$ T cells[6]. To elucidate the context-dependent role of Zbtb20 in immune regulation, tumor implantation experiments across multiple age groups are needed to better define how age influences the pro- or anti-inflammatory functions of Zbtb20 in the TME. In the EAE model, Zbtb20 appears to enhance CNS pathology by sustaining $CD4^+$ T cell activation and residency, potentially through IL-10–independent pathways. Prior studies indicate that Zbtb20 promotes prolactin-mediated expansion of $Eomes^+$ $CD4^+$ T cells, which drive chronic neuroinflammation[7]. Our data reinforce this notion, as WT mice exhibited more severe disease and greater $CD4^+$ T cell infiltration into the CNS. Future studies should profile cytokine levels in serum and CNS tissues at both acute and chronic stages of EAE to identify Zbtb20-specific immunological signatures. Moreover, the use of Zbtb20-reporter mice would enable in vivo tracking of $Zbtb20^+$ T cells during disease progression, offering a window into their dynamics in the TME and CNS.

References:

1. Beaulieu AM, Sant'Angelo DB. The BTB-ZF Family of Transcription Factors: Key Regulators of Lineage Commitment and Effector Function Development in the Immune System. The Journal of Immunology. 2011;187(6):2841–2847. https://doi.org/10.4049/jimmunol.1004006

2. Krzyzanowska AK et al. Zbtb20 identifies and controls a thymus-derived population of regulatory T cells that play a role in intestinal homeostasis. Science Immunology. 2022;7(71):eabf3717. https://doi.org/10.1126/sciimmunol.abf3717

3. Emmerich J et al. IL-10 Directly Activates and Expands Tumor-Resident CD8+ T Cells without De Novo Infiltration from Secondary Lymphoid Organs. Cancer Research. 2012;72(14):3570–3581. https://doi.org/10.1158/0008-5472.CAN-12-0721

4. Strle K et al. Interleukin-10 in the brain. Critical Reviews in Immunology. 2001;21(5):427–449.

5. Glatigny S, Bettelli E. Experimental Autoimmune Encephalomyelitis (EAE) as Animal Models of Multiple Sclerosis (MS). Cold Spring Harbor Perspectives in Medicine. 2018;8(11):a028977. https://doi.org/10.1101/cshperspect.a028977

6. Sankaran DG et al. Gene Regulatory Programs that Specify Age-Related Differences during Thymocyte Development. 2024 [accessed 2025 Mar 31]:2024.06.14.599011. https://www.biorxiv.org/content/10.1101/2024.06.14.599011v1. https://doi.org/10.1101/2024.06.14.599011

7. Zhang C et al. Extrapituitary prolactin promotes generation of Eomes-positive helper T cells mediating neuroinflammation. Proceedings of the National Academy of Sciences of the United States of America. 2019;116(42):21131–21139. https://doi.org/10.1073/pnas.1906438116

Preferential reliance on glycolytic metabolism by liver ILC1s

Pooja N. Kayala

Mentor: Dr. Aimee Beaulieu

Poster #

4th year Infection, Immunity, Inflammation (III): Ph.D. Candidate

Center for Immunity and Inflammation

The liver, an organ with important roles in host metabolism and defense, is home to unique liver-resident immune populations, including Type 1 "helper" innate lymphoid cells (ILC1s) ILC1s. Liver ILC1s are phenotypically and functionally similar to classic NK cells (cNKs) but are a separate innate lymphocyte lineage with distinct roles in memory responses, protection against liver injury, and control of tumors in the liver. Although cNKs are known to rely on glucose-fueled oxidative phosphorylation (OXPHOS), the metabolic dependencies of liver ILC1s remain poorly understood. Here, we demonstrate that liver ILC1s and cNKs are metabolically distinct cells that differ significantly in glucose uptake, mitochondrial content and polarization, and metabolic dependencies. Compared to liver cNKs, liver ILC1s import more glucose as seen by glucose-probe uptake assay. Moreover, using the SCENITH assay, we show that ILC1s preferentially rely on glycolytic versus OXPHOS-based metabolism, and have fewer polarized Mitotracker CMXros-positive mitochondria. Activation with pro-inflammatory cytokines or through activating receptor crosslinking increased glucose uptake by both liver ILC1s and cNKs, and activation-induced IFNγ production was dependent on glycolysis but not OXPHOS in liver ILC1s. Consistent with a preferential reliance on glucose-based metabolism, the expression of transcripts for the glucose transporters, GLUT1 and GLUT3, was higher in liver ILC1s compared to liver cNKs, and only liver ILC1s expressed transcripts for GLUT8. Future studies will investigate how glucose-based metabolism, including the use of specific glucose transporters, contributes to liver ILC1 effector and cytotoxic function in vivo. Ultimately, our study could identify novel metabolic pathways to therapeutically modulate ILC1 function in hepatic tumors, liver injury, and fibrosis.

Keywords: [Innate lymphoid cells I], [Natural killer cells], [Glucose metabolism], [Liver]

TLR4–MyD88–NF-κB signaling promotes *Acinetobacter baumannii* clearance in the airway via neutrophil recruitment

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Background: *Acinetobacter baumannii* is a multidrug-resistant Gram-negative pathogen that causes severe infections such as pneumonia and bacteremia, particularly in ICU settings. Its persistence in harsh environments and rapid acquisition of resistance genes have led to its classification as a "priority critical" pathogen by the WHO and a "serious threat" by the CDC. Despite increasing research interest, the host immune response to *A. baumannii*—especially within the airway—remains poorly understood. Airway epithelial cells form the first barrier against respiratory pathogens and shape early immune responses, yet their role in detecting and responding to *A. baumannii* infection is largely unexplored. Understanding these mechanisms is essential for identifying new strategies to enhance host defense and improve outcomes in infected patients.

Aims: The airway epithelium is a key component of the innate immune system, serving not only as a physical barrier but also as an active regulator of immune responses to respiratory pathogens. This research aimed to define how airway epithelial cells contribute to host defense against *A. baumannii*, with a focus on their role orchestrating early immune signaling. By identifying the key chemokines, receptors, and signaling pathways involved, this work sought to advance our understanding of the epithelial-immune interface in bacterial lung infection. Insights gained from this study may inform the development of host-directed therapies for combating multidrug-resistant *A. baumannii*.

Methods: We utilized multiple mouse infection models, including transgenic knockout and tissue-specific strains, to characterize the cells and molecular networks involved in the pathogenesis of *A. baumannii* infection. Gene expression analyses included qRT-PCR as well as comprehensive global transcriptomic profiling to capture broad changes in host signaling pathways. Immune cell recruitment was assessed by flow cytometry, while chemokines responses were quantified using multiplex cytokine analysis. Through this integrative approach, we deepened our understanding of the host response and identified immune components that contribute to limiting *A. baumannii* infection.

Results: Inhibition of NF-κB signaling, either pharmacologically or through airway epithelial cell-specific deletion of RelA, led to marked defects in bacterial clearance following *A. baumannii* infection. This defect was consistent across sexes and associated with significantly elevated bacterial burdens in both the bronchoalveolar lavage fluid and lung tissue. Further investigation revealed that airway epithelial NF-κB signaling was critical for early neutrophil recruitment, a key component of the innate immune response. At 4 hours post-infection, RelA-deficient mice exhibited substantial reductions in neutrophil numbers, coinciding with reduced expression and secretion of neutrophil-attracting chemokines such as G-CSF, GM-CSF, and CCL20.

Transcriptomic analysis of sorted airway epithelial cells confirmed that NF-κB signaling was required for the early induction of inflammatory and chemotactic programs during infection. To test whether reduced chemokine production accounted for the impaired clearance, exogenous administration of recombinant G-CSF, GM-CSF, and CCL20 restored early neutrophil recruitment and partially rescued bacterial clearance in RelA-deficient mice at later time points. Lastly, airway epithelial-specific deletion of MyD88 or TLR4 also impaired bacterial clearance, supporting the notion that the TLR4–MyD88–NF-κB signaling axis in airway epithelial cells is essential for orchestrating timely neutrophil recruitment and effective host defense against *A. baumannii* infection.

Conclusion: We found that inactivation of NF-κB, the innate sensor TLR4 and its adapter MyD88 within airway epithelial cells were required for clearance of *A. baumannii* from the airway. Inactivation of these components specifically in epithelial cells, impaired neutrophil recruitment and led to bacterial persistence. Exogenous administration of neutrophil chemokines restored early neutrophil influx, highlighting their role as downstream effectors. This work reveals epithelial-intrinsic innate signaling as a key determinant of host defense against multidrug-resistant pathogens.

ZBTB22 REGULATES CD8+ T CELL FUNCTION AND PERSISTENCE IN COLORECTAL CANCER

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Colorectal cancer (CRC) remains a leading cause of cancer-related mortality, with aging-associated immune dysfunction contributing to poor outcomes. CD8+ T cells play a critical role in anti-tumor immunity but become functionally impaired with age. We identified ZBTB22, a BTB-ZF transcription factor, as highly expressed in human memory CD8+ T cells, with its expression increasing in aged individuals. Unlike senescent T cells, ZBTB22+ CD8+ T cells retain proliferative and effector capacity, suggesting a role in maintaining CD8+ T cell function during aging. Since murine T cells lack endogenous *Zbtb22* expression, we developed a transgenic KI mouse model expressing human *ZBTB22* in T cells. This study investigates ZBTB22's role in regulating CD8+ T cell differentiation and function in response to CRC.

We implanted MC38 CRC cells into ZBTB22-expressing and control mice and assessed tumor growth and immune infiltration. Tumors in ZBTB22-expressing mice grew slower. Flow cytometry showed a decrease in PD-1 expression and an increase in Eomes+ CD8+ tumor-infiltrating lymphocytes, suggesting reduced exhaustion and enhanced effector differentiation. Further cytometry analysis of tumor-draining lymph nodes showed higher CD8+ T cell accumulation, suggesting improved priming or trafficking. Future adoptive transfer experiments will assess the role of ZBTB22+ CD8+ T cells in tumor control.

These findings indicate that ZBTB22 may enhance CD8+ T cell persistence and limit exhaustion in response to CRC, making it a potential tool for improving immunotherapy. Future studies will define its role in modulating anti-tumor immunity and explore its translational potential in human CRC.

Long-chain fatty acid sensing by GPR132 regulates CD8$^+$ T cell responses to infection

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CD8$^+$ T cells undergo robust expansion upon priming to control pathogen replication and provide host immunity; however, T cell function must be tightly regulated to ensure effective immunosurveillance without immunopathology. G protein coupled receptors sense environmental signals and are essential for modulating T cell function, and the lipid sensing receptor GPR132 is rapidly upregulated by T cells upon activation and maintained after infection is resolved. Ligands for this receptor include oxidized long-chain fatty acids derived from dietary linoleic acid, and these are increased under proinflammatory conditions. Additionally, mimics of these ligands are synthesized by commensal and pathogenic microorganisms. GPR132-deficient (KO) mice develop an autoimmune syndrome accompanied by expansion of the T cell compartment, suggesting GPR132 as a key target for T cell regulation. We examined the cell-intrinsic role of GPR132 in CD8$^+$ T cell responses during infection and found that KO T cells displayed significantly enhanced expansion over wild-type (WT) cells. However, WT and KO T cells had similar proliferation and gene expression profiles upon TCR-engagement *in vitro*. There were also no GPR132-dependent changes in T cell expansion observed during lymphopenia-driven proliferation *in vivo*, or when GPR132KO mice were aged in a SPF facility, indicating GPR132 inhibits T cell expansion specifically in the context of inflammation. An in-depth inquiry into the mechanism of action revealed that GPR132 limits T cell proliferation at peak of infection, while subsequently escalating cell death during contraction. Moreover, when T cells lacked GPR132 signaling, we observed an increase in the maintenance of CX3CR1$^+$ effectors. Transcriptomic analysis of splenic WT and KO antigen-specific CD8$^+$ T cells supported this finding, as GPR132 deficiency results in enlarged frequencies of cytotoxic effector cells at the expense of the memory compartment after infection. Additionally, formation of tissue-resident memory pools was diminished. GPR132 also regulated effector function, as granzyme production was reduced while cytokine production was increased in KO T cells. Lastly, despite increased numbers of KO memory T cells, they displayed reduced expansion potential as WT memory cells, yet retained enhanced proinflammatory cytokine production during rechallenge. Altogether, these studies reveal a role for GPR132 in detecting self and commensal lipids to regulate T cell responses against pathogens

and indicate that GPR132 can be targeted to modulate T cell number and function without induction of autoimmunity.

Abstract for Poster
13D Symposium Posterboard Session
Marmut, Eduard

Title:

Astrocytic RIPK3 exerts protective anti-inflammatory activity during viral encephalitis via induction of serpin protease inhibitors

Abstract:

Flaviviruses pose a significant threat to public health due to their ability to infect the central nervous system (CNS) and cause severe neurologic disease. Astrocytes play a crucial role in the pathogenesis of flavivirus encephalitis through their maintenance of blood-brain barrier (BBB) integrity and their modulation of immune cell recruitment and activation within the CNS. We have previously shown that receptor interacting protein kinase-3 (RIPK3) is a central coordinator of neuroinflammation during CNS viral infection, independent of its canonical function in inducing necroptotic cell death. However, roles for necroptosis-independent RIPK3 signaling in astrocytes are poorly understood. We used mouse genetic tools to induce astrocyte-specific deletion, overexpression, and chemogenetic activation of RIPK3 to demonstrate an unexpected function. Astrocytic RIPK3 signaling promoted neuroprotection via upregulation of serpins, endogenous protease inhibitors with immuno-modulatory activity including preservation of BBB integrity and reduction of leukocyte infiltration.

The E3-Ubiquitin ligase TRIM6 and Unanchored Ubiquitin Promotes Damaging Neutrophilic Inflammation During Viral Infection via the PI3K-AKT Pathway

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To avoid excessive inflammation, innate immune signaling is regulated by different posttranslational modifications, including the Ubiquitin system. We previously reported that the E3-ubiquitin ligase TRIM6 regulates antiviral type-I IFN responses. To evaluate the TRIM6-mediated immune signaling role *in vivo*, we generated *Trim6*$^{-/-}$ mice and challenged them with Influenza virus (IAV). As expected, *Trim6*$^{-/-}$ mice displayed higher viral titers in the lungs early during the infection. Surprisingly, the *Trim6*$^{-/-}$ mice exhibited reduced weight loss and increased survival kinetics compared to the *Trim6*$^{+/+}$ mice. Similar effects were observed with Ebola and SARS-CoV-2 infection in *Trim6*$^{-/-}$ mice. The infected *Trim6*$^{-/-}$ mice showed reduced levels of CXCL1 that correlated with decreased neutrophil infiltration into the lungs. Blocking the receptor of the CXCL1 signaling pathway using Reparaxin ameliorated disease in *Trim6*$^{+/+}$ but not *Trim6*$^{-/-}$ mice. This was also supported by a neutrophil depletion study using Lys6G in *Trim6*$^{+/+}$ mice. We hypothesize that TRIM6 promotes inflammation via CXCL1 and neutrophil recruitment. Using GFP-expressing IAV and single cell RNAseq we identified a lung fibroblast population that expresses TRIM6-dependent CXCL1 in non-infected cells. Mechanistically, TRIM6 knockout cells resulted in reduced AKT phosphorylation. Co-Immunoprecipitation assays and *in vitro* cell-free assays showed that TRIM6 interacts with the components of PI3K-AKT pathway and unanchored K48-polyubiquitin chains produced by TRIM6 promote dissociation of the p85 subunit of PI3K favoring the formation of p85 homodimers, enhancing its

lipid kinase activity. In conclusion, our study uncovers a novel mechanism by which the host cell regulates pathogenic CXCL1 expression and neutrophil recruitment during viral infection. Blocking CXCL1 signaling early during infection may provide a therapeutic strategy to promote host survival against highly pathogenic respiratory viruses.

Title: Integrated DNA-methylome and Transcriptome Signatures Identify Disrupted Developmental Pathways by Prenatal Smoke Exposure

Collaborators: Janaki Ramya Namburu, Priyadarshini Kachroo, PhD, Anoushka Basu, Gbenga Dairo, Alvin T. Kho, PhD, Kelan G. Tantisira, MD, Scott T. Weiss, MD, Dawn L. DeMeo, MD

Background: Maternal health during pregnancy plays a crucial role in fetal lung development, with in-utero smoke exposure (IUS-exposure) potentially impairing lung maturation and long-term respiratory health.

Purpose: Prenatal smoke exposure has long been associated with alterations in DNA methylation and gene expression. However, the precise impact of this intricate relationship in the context of IUS-exposure is yet to be fully understood. We aimed to investigate IUS-exposure as a modifier of methylation and functional gene expression pathways during lung development.

Methods: Our analysis integrated genome-wide DNA methylation (Illumina Infinium Human Methylation 850k BeadChip) and gene expression (Affymetrix GeneChip) profiles from 270 fetal lung tissue samples (154 IUS-exposed, 116 unexposed) to explore the molecular impact of IUS-exposure. Variance filtering and differential analysis using multivariate limma models were performed independently on each omics layer (with IUS-exposure as predictor and DNA methylation or gene expression as outcome) to identify IUS-associated features with overlapping gene annotations. These features were incorporated into the DIABLO framework (mixOmics), a supervised multiblock PLS-DA method, to identify multi-omics signatures predictive of IUS-exposure. Model performance was assessed using balanced error rate (BER), area under the curve (AUC), and inter-feature correlations.

Results: Following preprocessing, 49 CpG sites and 40 genes were selected for downstream integration using the DIABLO framework, with IUS-exposure modeled as a binary classification variable. Methylation data demonstrated stronger discriminatory capacity (AUC = 0.855 for Component 2) than gene expression data (AUC = 0.648), highlighting the greater epigenetic sensitivity to prenatal smoke exposure. Component correlation analysis revealed moderate cross-omics concordance (r = 0.67) on Component 2, supporting the integrative relevance of the selected features. Visualization techniques, including correlation circos and network plots, identified more inverse relationships between methylation and gene expression, consistent with transcriptional repression via hypermethylation. Clustered image maps showed improved group separation in Component 2, suggestive of exposure-linked coordinated hypomethylation and gene upregulation in genes such as *PTPRC*, *HLA-DQB1*, and *SLURP2*. Additional genes, including *RPS6KA2*, *CFH*, and *NID2*, are involved in oxidative stress response, immune modulation, and lung development, suggesting a potential epigenetic mechanism underlying IUS-induced developmental alterations.

Conclusion: Our findings reveal that IUS-exposure may disrupt epigenetic and transcriptional regulation linked to immune function, oxygen uptake, and extracellular matrix organization in early lung development. Functional validation of potential biomarkers would usher in new insights into fetal programming of smoking-related risk in lung diseases.

Analysis of hosts protein ubiquitination during Ebolavirus infection.

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Ebola virus (EBOV, family *Filoviridae*) is a highly pathogenic negative sense single stranded RNA virus that causes Ebola virus disease. Ubiquitination is a post translational modification of proteins that can play both proviral and antiviral roles, via directly regulating virus replication or by modulating host signaling pathways. Although there are previous reports of ubiquitination of cellular and viral proteins during EBOV infection, much about the modulation of this process during the viral replication cycle has not been described in depth. In this work, we analyzed the ubiquitination landscape of EBOV infected THP-1 monocytes and primary Renal Proximal Tube Epithelial Cell (RPTEC) by Mass Spectrometry (MS), to determine cell type-specific regulatory pathways involving ubiquitination of viral and/or cellular proteins. To compare innate immune inflammatory pathways, we used WT and a recombinant EBOV VP35 mutant virus (EBOV-VP35m) with reduced IFN antagonist activity. As expected, we observed an increased in IFN-stimulated genes (ISGs) upon infection with the EBOV-VP35m as compared to WT virus. We also found differential ubiquitination patterns between the different cell types and the two different viruses. Specifically, upon virus infection, we identified new ubiquitination sites on Vimentin, a cytoskeleton protein that can be involved in innate immune response and inflammasome activation. We validated Vimentin ubiquitination using K-to-R mutants and a transcriptionally active VLP system (trVLP). We also found increased ubiquitination of VAMP8, a SNARE protein involved in antiviral innate immune response. We hypothesize that ubiquitination on VAMP8 by TRIM6 may modulate VP35 mediated virus replication or innate inflammatory responses.

Deciphering the Role of *Zbtb20*+ T-cells in the Development and Progression of Type 1 Diabetes

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Uncontrolled inflammation is the cause of nearly every autoimmune disease, including type 1 diabetes (T1D)[1]. In T1D patients, insulin producing beta cells are killed when effector T-cells infiltrate the pancreas. Previous work in the lab has shown that there is a subset of regulatory T-cells (Tregs) that are identified and controlled by the master regulator BTB-ZF transcription factor *Zbtb20*. These *Zbtb20*+ T-cells were shown to have an essential role for maintaining intestinal homeostasis and preventing inflammatory bowel disease (IBD)[2]. These studies showed that it is possible to target this specific T-cell type to prevent the inflammation associated with autoimmunity. With the results seen for inflammation in the intestine, we hypothesize that these same *Zbtb20*+ T-cells will have an impact on preventing T1D onset and islet specific inflammation. To test this, we have developed a non-obese diabetic (NOD) reporter mouse that expresses GFP with *Zbtb20*. This NOD reporter mouse allows us to easily detect the *Zbtb20*+ cells and analyze their expression profiles using FACs. We can compare the *Zbtb20*+ cells in the NOD reporter to those of a B6 reporter mouse. Additionally, we can compare *Zbtb20*+ cells in a NOD mouse that has become diabetic to a NOD mouse that is not diabetic. Looking at the *Zbtb20*+ T and B-cells, we have seen that there are distinct expression profiles of the NOD mice vs the B6 mice. Additionally, we observed a difference in the frequency of *Zbtb20*+ CD4+T-cells and count of *Zbtb20*+ B-cells when comparing a diabetic NOD mouse to a non-diabetic NOD mouse. These comparisons give us insight into what role the *Zbtb20*+ cells play in the development and progression of T1D.

References:

1. Bluestone JA, Buckner JH, Herold KC. Immunotherapy: Building a bridge to a cure for type 1 diabetes. Science. 2021;373(6554):510-6. Epub 2021/07/31. doi: 10.1126/science.abh1654. PubMed PMID: 34326232.

2. Krzyzanowska AK, Haynes Ii RAH, Kovalovsky D, Lin HC, Osorio L, Edelblum KL, Corcoran LM, Rabson AB, Denzin LK, Sant'Angelo DB. Zbtb20 identifies and controls a thymus-derived population of regulatory T cells that play a role in intestinal homeostasis. Sci Immunol. 2022;7(71):eabf3717. Epub 2022/05/07. doi: 10.1126/sciimmunol.abf3717. PubMed PMID: 35522722.

Role of G9a in NK cell proliferation and metabolism

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Natural killer (NK) cells are innate lymphoid cells that actively monitor and eliminate viral pathogens and cancer cells. NK cell differentiation is associated with changes in proliferative, functional, and metabolic activity. NK cell differentiation in mice can be tracked based on the expression of CD27 and CD11b, with CD27+CD11b- cells representing less differentiated and CD27-CD11b+ cells representing more differentiated NK cell subsets. Data from our lab demonstrate that the histone methyltransferase, G9a, is an important negative regulator of NK cell differentiation. G9a-deficient NK cells exhibit a hypermature phenotype, with associated changes in gene expression. Here, we investigate the impact of G9a-deficiency on the proliferative and metabolic profiles of NK cells, and determine its activities reflect a role in chromatin remodeling. Using CellTrace Violet dye dilution assays, we demonstrate that G9a-deficient NK cells proliferate less after IL-15, both as a bulk population and when individual NK cell maturation subsets are analyzed. Our preliminary data also suggest that G9a-deficient NK cells may have more mitochondria than G9a-sufficient NK cells, although we did not observe significant differences in mitochondrial potential or neutral lipid content. Overall, our data suggest a critical role of G9a in regulating NK cell proliferation and a possible role in the regulation of metabolism in NK cells.

SARS-CoV-2 NSP14 Induces Internal m7G RNA Modifications and Alters Host Transcriptome

SARS-CoV-2, the causative agent of COVID-19, remains a persistent health concern. Beyond its well-characterized biology, the mechanisms by which the virus hijacks the RNA metabolism of host cells are yet to be determined. RNA modifications, such as N6-methyladenosine (m6A) and 5-methylcytosine (m5C), are known regulators of mRNA stability and translation, and internal N7-methylguanosine (m7G) modification has recently emerged as an important modulator of RNA processing. In this study, we investigated the role of the SARS-CoV-2 nonstructural protein 14 (NSP14) in inducing internal m7G modifications on host mRNAs. Overexpression of NSP14 in human cells significantly increased mRNA m7G levels, as detected by mass spectrometry and immunoassays. Importantly, this effect depends on NSP14's N7-methyltransferase activity, suggesting a critical catalytic role, and is enhanced when NSP14 forms a complex with the viral protein NSP10. Similar patterns were observed in other coronaviruses, suggesting a conserved role among these viruses. Transcriptomic analyses revealed that NSP14-induced m7G modifications correlate with increased intron retention and alternative splicing patterns, suggesting that internal m7G modification reshapes host gene expression. Our results demonstrate that SARS-CoV-2 NSP14 can alter the host transcriptome through RNA internal methylation.

Comparative Structural Evaluation of Ebola virus GP-Receptor Interactions Across Viral Species and Host Tropism Implications

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Filoviruses include highly pathogenic viruses that cause severe disease in humans with high mortality rates, including Ebola virus (EBOV), Sudan (SUDV), Bundibugyo (BDBV), Tai Forest (TAFV), and the non-pathogenic Reston (RESTV). Viral entry into host cells is mediated by the filovirus glycoprotein (GP), which is known to interact with receptors such as TIM-1, DC-SIGN, L-SIGN, and the intracellular receptor NPC1. While NPC1 has been structurally resolved in complex with EBOV GP, the others have only their GP-binding domains structurally characterized. We identified MRC1, which contains carbohydrate recognition domains (CRDs) homologous to those in DC-SIGN and L-SIGN, as a novel candidate receptor for EBOV. Using HDOCK, we modeled interactions between different filovirus GPs and TIM-1, DC-SIGN, L-SIGN, and the CRDs of MRC1. With PyRosetta and Surfaces, we estimated binding affinities and per-residue contributions. To explore therapeutic avenues, we performed high-throughput docking of FDA-approved compounds against GP using NRGRank and FlexAID. According to the docking models, MRC1 CRDs can bind GP significantly stronger than the known receptors. Consistent with recent experimental data, NPC1 interactions remained conserved across filovirus species. In contrast, receptor interactions involving TIM-1, DC-SIGN, L-SIGN, and MRC1 exhibited species-specific differences. Notably, TAFV showed reduced general affinity for many evaluated receptors, suggesting a mechanistic basis for its lower pathogenicity in humans. Regarding inhibitors, for NPC1, the known interaction inhibitor Clomifene emerged among top candidates, validating our approach. We also describe promising hits for other receptors. Our results support the hypothesis that MRC1 may function as a novel filovirus receptor. The applied methodology shows promise for elucidating species-specific differences in host tropism and for identifying small molecules capable of modulating these interactions. Ongoing efforts aim to validate inhibitors and expand modeling to receptors from additional hosts, offering further insight into filovirus transmission dynamics and cross-species infection risk.

Structure Based Drug Discovery Targeting Polymyxin Antibiotic Resistance

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Last-resort antibiotics, such as polymyxins, are paramount in the treatment of multi-drug resistant Gram-negative (GN) bacterial infections. Polymyxins exert their antimicrobial effect by associating with the negatively charged phosphate moieties of Lipid A, the lipid anchor of bacterial lipopolysaccharide, disrupting membrane integrity via a detergent-like mode of action. Despite their efficacy, resistance to polymyxins has been observed in several pathogenic Enterobacteriaceae, such as *S. enterica*, via covalent modification of Lipid A with cationic moieties such as 4-amino-4-deoxy-α-L-arabinopyranose (L-Ara4N). In GN bacteria, modification of Lipid A with L-Ara4N is facilitated by an enzymatic relay of eight proteins collectively referred to as the aminoarabinose biosynthetic pathway (ABP). Of these eight proteins, membrane aminoarabinose transferase, or ArnT, is the enzyme responsible for catalyzing the last step of the ABP in which aminoarabinose is transferred from undecaprenyl-phosphate to Lipid A. ArnT serves as an attractive target for the development of therapeutic agents capable of reversing polymyxin resistance in GN bacterial species. For this reason, we have decided to investigate the mechanics of substrate binding in *S. Enterica* ArnT ($ArnT_{Se}$). Here we share the results of molecular dynamics simulations in which a substrate-bound cryo-EM structure of $ArnT_{Se}$ was subjected to free energy calculations, revealing a number of residues involved in substrate binding. We validate these computational findings in-vitro using site-directed alanine mutagenesis coupled with a bacterial growth inhibition assay, revealing key residues that are essential to ArnT's function and its ability to confer polymyxin resistance. We also share preliminary results of structure based virtual drug screening. Overall, these results provide further insight into the structural and mechanistic attributes of ArnT and enhance our understanding of this enzyme as a potential drug target.

The Role of Post-Translational Modifications During *Ebola virus* Induced Immune Dysregulation

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Zaire Ebola virus (EBOV) infection induces a cytokine storm by dysregulating the immune response, causing immunopathology and disease. Although the cytokine storm is the hallmark of EBOV infection and disease, the immune signaling pathways and regulatory mechanisms targeted during EBOV infection remain incompletely defined. We joined a collaborative effort to characterize the signaling pathways on all levels of regulation, from DNA-to-RNA-to-protein so we can construct a complete picture of how EBOV dysregulates the immune response. Our role focuses on post-translational modification (PTM) enzymes, identifying how their expression and activity regulate key on and off signals for the immune response. Because phosphorylation is known to regulate many cytokines and chemonkines, we analyzed mass spectrometry data of EBOV infected mouse bone marrow derived dendritic cells to identify phosphatases with differential expression over the course of viral infection. Protein Tyrosine Phosphatase Nonreceptor Type 13 (PTPN13) was identified in our data due to its decreased expression during EBOV infection. PTPN13 KO resulted in increased Interferon beta (IFNβ) production through increased phosphorylation of Interferon Regulatory Factor 3 (IRF3). Co-immunoprecipitation revealed that PTPN13 and IRF3 interact, with the interaction being stronger when IRF3 is phosphorylated. Currently, we are investigating a tyrosine residue on IRF3 to determine if this site is targeted by PTPN13 for dephosphorylation and influences IRF3 activation. When we infected PTPN13 KO cells with EBOV, we observed a slightly higher level of virus titers early in infection and an overall decrease in interferon stimulated genes (ISGs) and immune signaling, indicating that PTPN13's increase in IFN signaling does not overcome EBOV's IFN antagonism. Additionally, we found that PTPN13 KO significantly decreases basal levels of AKT phosphorylation, decreasing Tumor Necrosis Factor signaling in mock and EBOV infected lung epithelial cells. We are currently still investigating how these mechanisms piece together and result in enhanced EBOV infection and immune dysregulation leading to disease.

Keywords: Innate immunity, Virus-host interactions, Virus interference with host defense

New Instrumentation and Services at the Center for Advanced Proteomics Research: Advancing Immune and Metabolic Research in Pathogen Responses

The Center for Advanced Proteomics Research (CAPR) has expanded its analytical capabilities with the recent acquisition of two state-of-the-art mass spectrometry platforms: the **Waters Desorption Electrospray Ionization (DESI) Mass Spectrometer** for spatial metabolomics and the **Bruker timsTOF Ultra 2 Mass Spectrometer** for high sensitivity immunopeptidomics. These advanced technologies provide crucial tools to investigate how immune and metabolic pathways respond to pathogenic challenges at an unprecedented level of detail.

The **Waters DESI MS** enables label-free, high-resolution **spatial metabolite imaging**, preserving tissue integrity while mapping metabolic alterations in response to infection. This technology provides key insights into metabolic adaptations within infected tissues, immune-metabolism interactions, and host-pathogen metabolic dynamics, facilitating biomarker discovery and therapeutic targeting.

The **Bruker timsTOF Ultra 2** offers enhanced **trapped ion mobility spectrometry (TIMS) with parallel accumulation serial fragmentation (PASEF)**, significantly improving sensitivity and throughput for **immunopeptidomics**. This platform enables comprehensive profiling of pathogen-derived peptides presented by major histocompatibility complex (MHC) molecules, providing insights into antigen processing and immune recognition critical for vaccine and immunotherapy development.

These new capabilities at CAPR will advance research on pathogen-host interactions by providing **high-resolution spatial metabolomics and in-depth immunopeptidomics**, essential for understanding immune responses, metabolic adaptations, and pathogen evasion strategies. We welcome collaborations and look forward to supporting researchers studying immune and metabolic responses to pathogens with these powerful new analytical services.

Metabolic Reprogramming of Host Immunity During Tuberculosis: Pathways to Host-Directed Therapies

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Abstract: The field of immunometabolism in tuberculosis (TB) is uncovering new strategies for host-directed therapies (HDTs). Through comprehensive analyses including metabolomics and single molecule RNA-FISH, we have identified key metabolic pathways and mTORC1 signaling that are activated in immune cells during Mycobacterium tuberculosis (Mtb) infection. In addition to increased glycolysis, there is enhanced arginine metabolism, activation of glutaminolysis, and glycolysis-driven serine synthesis, followed by its breakdown in the mitochondrial folate cycle. These findings underscore the central role of amino acid and mitochondrial metabolism in supporting immune defenses. Isotope tracing studies in Mtb-infected macrophages reveal that glutaminolysis fuels both the oxidative and reductive TCA cycles, generating key signaling and antimicrobial molecules (e.g., succinate, itaconate) and biosynthetic precursors such as aspartate. In vivo, glutamine supplementation reduces Mtb burden, mitigates lung damage, and improves host health during chronic infection by enhancing antimicrobial responses via the activation of key metabolic pathways and mTORC1 signaling. These insights suggest that targeting amino acid and mitochondrial metabolism offers a novel therapeutic approach for HDTs in TB management.

EGFR biased signaling in intestinal health and disease

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Despite the long history of EGFR inhibition in cancer therapy, much is still unknown about EGFR in regulating intestinal homeostasis. EGFR regulates epithelial regeneration in the intestine and macrophage inflammatory function during inflammatory bowel disease (IBD). Multiple ligands bind to EGFR, but EREG and EPIGEN induce a unique signaling pattern marked by weak EGFR dimerization and sustained Erk signaling, which leads to cellular differentiation in vitro. This phenomenon, in which a single receptor induces distinct signaling depending on the identity of the ligand, is termed 'biased signaling'. The role of EGFR biased signaling in intestinal homeostasis and IBD is currently unknown.

The R84K point mutation on EGFR, originally identified in glioblastoma patients, was found to eliminate EGFR biased signaling *in vitro*. To examine the biological impact of EGFR biased signaling *in vivo*, we generated $EGFR^{R84K}$ mutant mice, which have a phenotypically healthy intestine and healthy overall appearance. We found that the $EGFR^{R84K}$ mice are protected against dextran sodium sulfate (DSS)-induced colitis. Preliminary bone marrow transplantation experiments suggest that the $EGFR^{R84K}$ signaling within the non-hematopoietic cells may drive the protection against colitis, while the $EGFR^{R84K}$ signaling within the hematopoietic cells may worsen the DSS-induced colitis. Further, assessing the intestinal epithelial regeneration after irradiation, showed decreased expression of inflammatory markers in $EGFR^{R84K}$. Thus, irradiation and colitis models both exhibit protection by $EGFR^{R84K}$. To understand whether microbiome dysbiosis may play a role in this protection, we used fecal microbiome transplant experiments in gnotobiotic mice. Surprisingly, we find that $EGFR^{R84K}$ FMT worsens DSS colitis, suggesting the $EGFR^{R84K}$ mechanism of protection is host-intrinsic, and functions despite microbiome dysbiosis.

Altogether, our ongoing studies aim to provide mechanistic understanding on how the EGFR biased signaling impacts intestinal health, and may facilitate the development of EGFR-targeted therapeutics which support the repair of the intestinal mucosa, without leading to cancer; which may be of particular interest for chronic inflammatory diseases such as IBD.

Keywords: Inflammatory Bowel Disease, EGFR, Intestinal Epithelial Cells, Myeloid Cells, Epithelial regeneration.

Tfh cell regulation and the impact on B cells in viral infection

Eden Hirsch
Jason Weinstein, PhD

$CD4^+$ T cells and B cells are key players in adaptive immune responses, providing long-lasting protection against pathogens through cytokine and antibody production. Thus, it is necessary to understand the cellular mechanisms that produce antibodies to better treat infection and enhance immunization. Since these immune cells are crucial for successful protection, regulating this response is key to preventing overstimulation and autoimmunity. This research aims to understand the mechanisms by which natural killer (NK) cells modulate $CD4^+$ T and B cells, thereby altering the antibody response during viral infection, and how that may differ in autoimmune disorders. A subset of $CD4^+$ T cells, T follicular helper (Tfh) cells, and B cells, germinal center (GC) B cells, are critical for generating antibody responses during viral infections and autoimmunity. Previous studies in viral infections have implicated NK cells as key regulators of Tfh and GC B cell responses. We have confirmed that NK cells regulate Tfh cells and GC B cells, but have expanded these findings to show that a newly discovered B cell subset, $Tbet^+$ $CD11c^+$ B cells (tBCs), are also impacted by NK cells. Here, we utilize both an antibody depletion and a novel NK cell knockout model to gain insight into the interactions between NK and Tfh cells and the effects on the B cell response. Following acute viral infection, we find that Tfh cells, GC B cells, and tBCs increase in the absence of NK cells. Importantly, the inducible costimulator (ICOS), an essential activation receptor for Tfh cell development, is highly expressed in these cells upon NK cell depletion. When we analyzed NK cells throughout viral infection, we also saw upregulation of ICOS ligand (ICOSL) by NK cells, indicating the interaction between these cell types could be ICOS-dependent. Together, these data indicate that NK cells may regulate overactivated Tfh cells in viral infection through ICOS-ICOSL interactions, thus altering the B cell response.

Authors:
Paul Brennan, Darin Wiesner

PROJECT TITLE
Characterization of the Pulmonary Arteriole as a Portal of Entry for Rapid Neutrophil Influx to *Coccidioides* Spore-Exposed Terminal Bronchioles.

PROJECT ABSTRACT
Rapid neutrophil influx into the lung is essential for protecting against inhaled pathogens like *Coccidioides* fungal spores, which cause over 350,000 infections annually in the United States. Numerous studies have uncovered mechanisms for neutrophil influx into terminal alveolar spaces, but fungal spores often deposit within the more proximal conducting airways of the lung. Some work has shown that the systemic circulation supporting bronchi can support this recruitment function, but the terminal bronchiole, where we observe *Coccidioides* spore deposition and resulting inflammation, is too far down the bronchial tree to receive this bronchial circulation. Utilizing thick (1mm) precision-cut lung slices (PCLS), we have discovered that the terminal bronchiole is a transitional zone within the lung where a primary source of recruited neutrophils is the pulmonary arteriole. This vessel is a previously uncharacterized site of leukocyte infiltration in the lung. We have discovered that VCAM1 is rapidly upregulated by arteriolar endothelium following infection, and the monoclonal blockade of VCAM1 significantly reduces number of neutrophils able to migrate into the lung interstitium and airway spaces. While NFkB is a master regulator of inflammatory adhesion molecules including VCAM1, we show that VCAM1 upregulation in our model is NFkB independent. Our studies hope to elucidate a detailed mechanism of a previously unappreciated source of inflammation, the pulmonary arteriole, which may have implications in many acute and chronic inflammatory pulmonary pathologies.

IDO-dependent metabolic reprogramming promotes *K. pneumoniae* airway infection

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Klebsiella pneumoniae (Kp) is a leading cause of healthcare-associated pneumonia and often proves refractory to treatment, even when susceptible to antimicrobials. Kp infections are associated with a maladaptive immune response, marked by early accumulation of immunosuppressive myeloid cells such as M2-like macrophages and myeloid-derived suppressor cells (MDSCs), which are ineffective at clearing the bacteria. Our previous work demonstrated that central host metabolic pathways, including glutaminolysis and fatty acid oxidation (FAO), promote an immune response permissive to infection. We hypothesise that metabolic pathways beyond core metabolism also facilitate infection. Specifically, we propose that host tryptophan catabolism to the immunosuppressive metabolite kynurenine by the enzymes indoleamine 2,3-dioxygenase 1 and 2 (Ido1/Ido2) promotes MDR *Kp* persistence in the airway. Using a mouse pneumonia model in both BL/6 and *Ido1/2*$^{-/-}$ backgrounds, we compared bacterial burden, and immune response by flow cytometry in mice. Metabolites were visualized *in situ* using desorption electrospray ionization mass spectrometry (DESI-MS). We show that *Ido1/2*$^{-/-}$ is protected against pulmonary Kp infection, as compared with BL/6, with enhanced survival and significantly reduced bacterial burden. Interestingly, we observed an increase in monocytes, neutrophils and T cells in the airways in infected *Ido1/2*$^{-/-}$ mice. Infected *Ido1/2*$^{-/-}$ mice have increased AMP in lung tissues compared to BL/6 mice, suggesting the potential activation of AMPK, a major energy sensor and metabolic regulator. Our findings highlight the role of Ido1/Ido2 and cellular metabolism in promoting effective immune cell function during Kp infection. Our data suggest that targeting host metabolism could offer a promising alternative or adjunctive approach for treating recalcitrant infections.

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