Case Western Reserve University School of Medicine

Department of Pathology

18th Annual Immunology Retreat

and

The Masamichi Aikawa Memorial Lecture

Friday, April 18, 2025
Cleveland Botanical Garden

CASE WESTERN RESERVE UNIVERSITY
School of Medicine

Cleveland Clinic
Lerner Research Institute

University Hospitals
Cleveland Medical Center

Infectious Diseases
HIV Medicine
CWRU • UHCMC • VAMC

CFAR
CENTERS FOR AIDS RESEARCH
Case Western Reserve University
University Hospitals of Cleveland

TBRU
Tuberculosis Research Unit

Center for Global Health
& Diseases

Welcome and Introduction

Welcome!

Immunology has a long and storied history in Cleveland, including the discovery of the Alternative Pathway of complement activation. The Immunology Training PhD Program in the Department of Pathology at Case Western Reserve University School of Medicine has served as a central organizational focus through which many groups are brought together. These include the CWRU Department of Pathology, Cleveland Clinic Department of Inflammation and Immunity, CWRU Center for Global Health and Disease, the CWRU Center for AIDS Research, the CWRU Comprehensive Cancer Center, and the University Hospitals Cleveland Medical Center Division of Infectious Diseases, including the Tuberculosis Research Unit. The diversity among these groups provides a rich confluence of basic science and clinical resources, enriching the research and training of students, fellows, and faculty alike as they engage in cutting-edge research in the field of immunology.

This is the 18th Annual Immunology Retreat, which continues to provide a focus for the development of interdepartmental and inter-institutional collaborations, training grants, program project grants, and other collaborative programs that will enhance immunology research and training in our community. Through cooperation and common purpose, the hope is to bring together all local investigators interested in immunology regardless of programmatic affiliation.

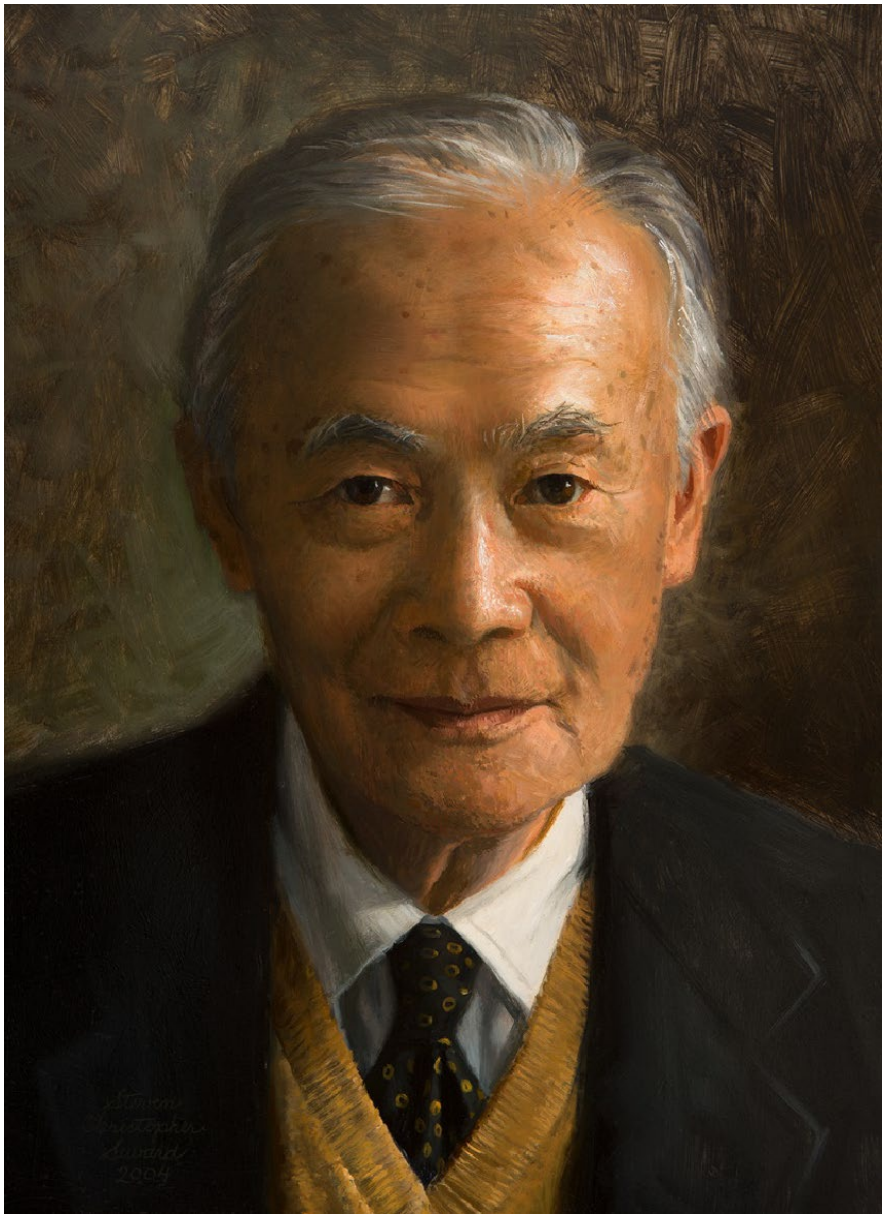
This year we have incorporated the Masamichi Aikawa Memorial Lecture into the program. Our Aikawa Lecturer this year is Dr. Chandy C. John, Distinguished Professor and Ryan White Professor of Pediatrics at Indiana University School of Medicine. Dr. John is a world-renowned expert on severe malaria, including neurodevelopmental impairment, transmission, and immunity, and we are excited to welcome him to Cleveland.

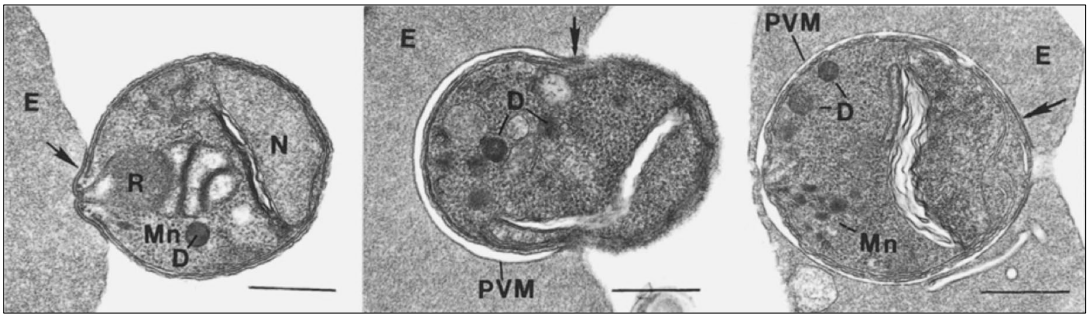
Finally, I would like to personally thank everyone on the planning committee: Drs. Wendy Goodman, Anna Bruchez, Anna Valujskikh, Michael Freeman, Marilia Cascalho, Stephanie Langel, and Theresa Pizarro. I'd also like to thank the administrative staff: Gail Stringer and Andrea Shellenberger for helping make the arrangements. I also need to thank all of the departments and divisions, especially Dr. Thad Stappenbeck for co-sponsoring this event. And of course, I need to thank our Department Chair and Cheerleader-in-Chief Dr. Cliff Harding, and all of our participants for making this event an outstanding way to spend a springtime Friday.

Thank you!

Brian A. Cobb, PhD
Director, Immunology Training Program

The 2025 Aikawa Memorial Lecture





Masamichi Aikawa, 1931 – 2004

Dr. Masamichi Aikawa received his MD degree from Kyoto University in 1958 and undertook his residency training in Pathology at Georgetown University Hospital, in Washington, DC. Between 1964 and 1968, he worked at Walter Reed Army Institute of Research, during which time he developed his reputation as an authority on the ultrastructure of malaria-causing Plasmodium species using electron microscopy. In 1968, Dr. Aikawa moved to CWRU and joined the Department of Pathology, retiring in 1995. In Cleveland, Dr. Aikawa married Hiroko and they raised their children, Keiko and Taro. Although he finished his career at Tokyo University, he remained a Professor Emeritus here at CWRU until his death in 2004. Dr. Aikawa was a part of many important discoveries surrounding Plasmodium biology. His iconic images showing red cell entry of the Plasmodium merozoite continue to guide the work of laboratories around the world attempting to develop a vaccine to reduce the public health threat of malaria.

Dr. Aikawa authored and coauthored over 340 articles and reviews during his distinguished 40-year career, many of which appeared in the most highly acclaimed scientific journals with broad readership. As a testament to the quality, originality and innovative nature of his work, Dr. Aikawa received the Alexander von Humboldt Research Award and was a visiting scholar at the Max Planck Institute of Biochemistry in 1984.

Tributes in Reminiscences of Masamichi Aikawa, published for the first Aikawa Memorial Lecture in 2005, recalled a thoughtful scientist who valued quality results, a generous and encouraging mentor and friendships that lasted decades and stretched beyond the laboratory and lecture hall.

Recent Aikawa Memorial Lecture Speakers

Thomas Nutman	2010
Fidel Zavala	2012
David Sibley	2014
Thomas Wellems	2015
Stefan Kappe	2016
Joseph DeRisi	2017
Terrie Taylor	2018
Alan Sher	2019
Chris King	2022
Sharon Hillier	2023

In grateful recognition of the Aikawa family and friends for their generosity in establishing and supporting the Masamichi Aikawa Memorial Lectureship endowment. This lectureship encourages excellence in structural biology and molecular parasitology and serves as a permanent tribute to our former faculty colleague in the Department of Pathology and Center for Global Health & Diseases at Case Western Reserve University School of Medicine.

2025 Masamichi Aikawa Memorial Lecture Speaker

Chandy C. John, MD
Distinguished Professor, Ryan White Professor of Pediatrics
Professor of Medicine, Professor of Microbiology & Immunology
Division Chief, Pediatric Infectious Diseases and Global Health
Indiana University School of Medicine
Indianapolis, IN, USA

Dr. John's research studies are based in Uganda and Kenya, in collaboration with Makerere University, the Kenya Medical Research Institute, and Moi University. His research has advanced scientific and medical understanding in four key areas: (1) Neurodevelopmental impairment in African children who have recovered from severe malaria, (2) Pathogenic mechanisms of severe malaria, (3) Malaria transmission and immunity, and (4) Reducing infection in African children with sickle cell anemia.

Dr. John's research has led to more than 260 peer-reviewed publications. He is an international leader in global child health and infectious diseases. He is a member of the American Academy of Pediatrics Committee on Infectious Diseases and has served on the Fogarty International Center Advisory Board, as co-chair of the Thrasher Research Fund Scientific Advisory Committee and as president of the American Society of Tropical Medicine and Hygiene.

Key awards received by Dr. John include election to the Association of American Physicians, the Caroline Breese Hall Award from the Pediatric Infectious Diseases Society, the Segar Family Award from the Midwest Society for Pediatric Research, the Bailey Ashford Medal from the American Society of Tropical Medicine and Hygiene, and the Pediatric Infectious Diseases Society Young Investigator Award.

Program Schedule

7:30 – 8:00am: Arrival / Check-in

8:00 – 8:30am: Continental Breakfast

8:30 – 10:00am: Oral Session I
Moderator: TBD

8:30 Julie Zhou (#58)
Intestinal stem cells enhance mucosal immunity through apoptotic body phagocytosis

8:45 Erik Koritzinsky (#23)
NK cell mediated allorecognition shapes reprogramming of neutrophils within heart allografts

9:00 Leighanne Main (#26)
The vaginal microbiome pre-vaccination associates with local and systemic HIV vaccine immune responses

9:15 Bailey Klein (#22)
Mast Cell Derived Histamine Negatively Regulates Hematopoiesis

9:30 Caitlin Snyder (#45)
Macrophage TRPV4 Drives Myofibroblast Differentiation through Activation of TGF-β

9:45 Vargab Baruah (#5)
HCMV Restricts the Innate Immune Response by Nuclear Export of Host Restriction Factor DDX41

10:00 – 10:30am: Poster Flash Talks
Moderator: TBD

10:00 Emily Blaum (#7)
The oral microbiome is dynamic during CAR T-cell therapy

10:02 Erica Orsini (#37)
NF-κB-Dependent Transcriptional Regulation of Piezo1 Mediates Bacterial Clearance of P. aeruginosa from the Lung

10:04 Jose Ignacio Valenzuela (#51)
Interleukin-27 Regulates B and T Cell Responses in Transplantation and Shapes Donor-Specific Alloantibody Production

10:06 Lwar Naing (#32)
TNFRSF13B Variants Modify Adaptive Immunity to Pathogens

10:08 Mayara Garcia de Mattos Barbosa (#4)
TNFRSF13B polymorphisms impact antibody effector properties determining allograft immunopathogenesis

10:10 Mikayla Ybarra (#56)
CAR T-Cell Resistance in Multiple Myeloma Using a Novel Human Bone Marrow Chip

10:12 Owen Meadows (#30)
Activation of the inflammatory response through IFN-γ treatment requires Krüppel-like factor 6 signaling

10:14 Pari Baker (#3)
Assessing milk transmission of bovine-origin H5N1 avian influenza virus in the lactating ferret model

10:16 Ruiting Zhou (#59)
STAT1 gain-of-function (GOF) mutations disrupt the conformational balance of STAT1 and lead to immunodeficiencies

10:18 Indrani Das (#12)
High-throughput compound screening reveals small molecule mediators of NK cytotoxicity against solid cancers

10:30 – 10:45am: Coffee Break

10:45 – 11:45am: Poster Session I

Abstract #	Presenter	Title
1	**Matthew Anderson**	CD8 cytotoxic T-cell infiltrates and cellular damage in the hypothalamus in human obesity patients
2	**Corynn Appolonia**	Sam68 is a critical regulator of retinal angiogenesis
3	**Pari Baker**	Assessing milk transmission of bovine-origin H5N1 avian influenza virus in the lactating ferret model
4	**Mayara Garcia de Mattos Barbosa**	TNFRSF13B polymorphisms impact antibody effector properties determining allograft immunopathogenesis

6	**Dijana Bjelivuk**	Long-acting IL-7 Induces Lymphoid Aggregates in the Tumor Microenvironment
7	**Emily Blaum**	The oral microbiome is dynamic during CAR T-cell therapy
8	**Adam Boulton**	Macrophage Mechanosensor Transient Receptor Potential Vanilloid 4 (TRPV4) and NF-κB Interact Inhibiting Bacterial Pneumonia-Induced Lung Injury
9	**Alyssia Broncano**	Nuclear estrogen receptors modulate CD4+ T cell responsiveness to TGFβ
10	**Gracie Carlson**	Inflammatory signaling in endothelial cells drives changes in IgG sialylation
12	**Indrani Das**	High-throughput compound screening reveals small molecule mediators of NK cytotoxicity against solid cancers
14	**Brayden Beathe-Gatheley**	A Strategy to Generate Anticipatory Immunity Against Mutable Viruses
15	**Megan Grund**	Transient Receptor Potential Vanilloid 4 (TRPV4) increases *Burkholderia cenocepacia* bacteria clearance in macrophages in vitro and lung infection in vivo via regulation of autophagy
16	**David Gurarie**	Agent-based modeling of parasite-immune malaria system with applications to data analysis, disease intervention, and evolution of drug resistance
17	**Jacob Ingber**	Insights into heterologous immunity: ELISpot analysis of diverse mouse stimulator-responder strain combinations
18	**Sidra Islam**	CRISPR-Based Kinome Screening to identify new kinase targets for lung cancer treatment
19	**Paul Karell**	Carbohydrate malabsorption as a potential mechanism underlying selective IgA deficiency
21	**Kayla Klatt**	Investigating the regulation and functions of PRSS1 and PRSS2 in natural killer cells
24	**Emily Kukan**	Loss of CD22 affects macrophage derivation and function
25	**Susana Lechuga**	Unconventional Myosin 18A is a novel regulator of the intestinal epithelial barrier and mucosal inflammation
27	**Rachel Martin**	Differential Proinflammatory Cytokine Expression and the Immune Response to Bacterial Vaginosis: the THRIVE Study
29	**Sarah McNeer**	Complement mediates strain-specific sensing and phagocytosis of *Debaryomyces hansenii*

30	**Owen Meadows**	Activation of the inflammatory response through IFN-γ treatment requires Krüppel-like factor 6 signaling
31	**Ashomathi Mollin**	Breastmilk Anti-Rotavirus Antibodies and Their Impact on Rotavirus Infection
32	**Lwar Naing**	TNFRSF13B Variants Modify Adaptive Immunity to Pathogens
48	**Vinicius Suzart**	Mycobacterium tuberculosis (Mtb)-infected cells are recognized by autologous memory CD4+ T cells

11:45 – 1pm: Lunch and Free Time to Explore Gardens

1:00 – 2:30pm: Oral Session II
Moderator: TBD

1:00 Blake McCourt (#28)
C9orf72 in myeloid cells prevents an inflammatory response to microbial glycogen

1:15 Daniel Kingsley (#20)
Osteosarcoma Utilizes VCAM1-VLA4 Signaling Axis on Macrophages to Promote Pulmonary Metastasis

1:30 Kaylynn Vidmar (#52)
Investigating the role of intestinal epithelial cell (IEC)-derived gasdermin C (GSDMC) in the regulation of IL-33 and its impact on chronic intestinal inflammation

1:45 Jordan Cress (#11)
WNK1 is a Novel Regulator of Aberrant Signaling in Acute Myeloid Leukemia

2:00 Daniel Feinberg (#13)
Oncostatin-M Ligand-Based CAR-T Therapy Targets and Eliminates Osteosarcoma

2:15 Avinaash Kaur Sandhu (#42)
Determining the genes and pathways in M2-like macrophages responsible for subverting CD4+ T-cell activation upon infection with Mycobacterium tuberculosis

2:30 – 2:45pm: Coffee Break

2:45 – 3:45pm: Poster Session II

Abstract #	Presenter	Title
33	**Namita Nanda**	IL7 increases stem-like CD8+T cells in the tumor microenvironment
34	**Kevin Newhall**	A specific clade of *Debaryomyces hansenii* is gut-adapted and induces distinct host immune responses
35	**Thu Nguyen**	TNFRSF13B polymorphism and maternal-fetal host defense
36	**Katelyn O'Hare**	Vascular Tissue Expression and Immune Recognition of Citrullinated Self-Proteins in People with HIV
37	**Erica Orsini**	NF-κB-Dependent Transcriptional Regulation of Piezo1 Mediates Bacterial Clearance of *P. aeruginosa* from the Lung
38	**Samuel Osanyinlusi**	Human cytomegalovirus G protein-coupled receptor (GPCR) UL78 regulates virus reactivation from latency
39	**Michelle Pan**	Induction of necroptosis releases MLKL's executioner domain before its RIPK3-mediated phosphorylation
40	**Lane Pierson**	Testing of Bat STING Orthologs Reveals Species Specific Differences in STING Functionality
41	**Michelle Raymond**	Myosin 18A regulates central and peripheral B cell tolerance
43	**Elizabeth Seidita**	TGFBRAP1 Overexpression Inhibits SARS-CoV-2 and Filovirus Entry by Modulating Endosomal Trafficking
44	**Priya Das Sinha**	Nitric oxide plays a crucial role in regulation of Cytochrome P450 activities by controlling the allocation of heme within the cell
46	**Lauren Stafford**	Harnessing breastmilk immunity to protect neonates against influenza virus
47	**Mamta Sumi**	Expression of soluble guanylate cyclase (sGC) and its ability to form a functional heterodimer are crucial for reviving the NO-sGC signaling in PAH
49	**Reyhaneh Tabatabaei**	The effect of altered B cell metabolism on vaccine response in obesity
50	**Abhirami Thumsi**	Inverse-vaccines for Rheumatoid Arthritis Re-establish Metabolic and Immunological Homeostasis in Joint Tissues
51	**Jose Ignacio Valenzuela**	Interleukin-27 Regulates B and T Cell Responses in Transplantation and Shapes Donor-Specific Alloantibody Production

53	**Hannah Wargo**	Gut Microbiome-Dependent Th17 Skewing Drives Sex Differences in a Murine Model of Crohn's Disease-like Ileitis
54	**Joseph Williams**	Differential gasdermin B (GSDMB) isoform usage and function in intestinal goblet cells
55	**Anna Winnicki**	Furthering our understanding of how human antibody epitopes on Apical Membrane Antigen 1 are related to clinical protection from *P. vivax* malaria: Preliminary data & study design
56	**Mikayla Ybarra**	CAR T-Cell Resistance in Multiple Myeloma Using a Novel Human Bone Marrow Chip
57	**Ziyin Zhao**	A Novel Bioinformatic Method to Trace Donor-specific B Cell Evolution in Transplant Recipients
59	**Ruiting Zhou**	STAT1 gain-of-function (GOF) mutations disrupt the conformational balance of STAT1 and lead to immunodeficiencies

4:00 – 5:00pm: **Aikawa Memorial Lecture**
Dr. Chandy C. John
Distinguished Professor, Ryan White Professor of Pediatrics
Professor of Medicine, Professor of Microbiology & Immunology
Division Chief, Pediatric Infectious Diseases and Global Health
Indiana University School of Medicine

5:00 – 5:30pm: **Closing Remarks and Awards**

Abstracts

Abstract 1

CD8 cytotoxic T-cell infiltrates and cellular damage in the hypothalamus in human obesity patients

Matthew P. Anderson[1-4*], Jared T. Ahrendsen[1-2], Yi Nong[1-4], Yuda Huo[1-2], Jasmine Steele[1-2]

[1]Beth Israel Deaconess Medical Center and [2]Harvard Medical School Boston MA
[3]Oxford-Harrington Rare Disease Center, University Hospitals and [4]Case Western Reserve University, Cleveland, OH

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Rare cases of paraneoplastic obesity in children suggest sporadic obesity might arise from adaptive immune cell-mediated mechanisms. We quantified lymphocytic inflammation in hypothalamus, a central regulator of feeding behavior and energy expenditure, and other brain regions in a cohort of obese and non-obese human post-mortem brains. Here we report that CD3 and CD8-positive (cytotoxic) T cells are increased in hypothalamic median eminence/arcuate nucleus (ME/Arc) and bed nucleus of the stria terminalis (BNST) in 40% of obese ≥ 30 BMI (body mass index) compared to non-obese patients. T cells were not increased in other brain regions. T-cells were most abundant in individuals with concurrent obesity and diabetes. Markers of cytotoxic T-cell induced cellular damage, activated caspase 3 and poly-ADP ribose, were also elevated in the ME/Arc of obese patients. To test if the induction of T-cell infiltration into ME/Arc of mice could trigger body weight gain we performed stereotaxic injections of immunogenic green fluorescent protein expressed by adeno-associated virus (AAV-CMV-GFP) or saline. AAV but not saline injections caused a gradual and significant weight gain until they were sacrificed 6 weeks after injection. CD8 T-cells were also increased in ME/Arc in mice with AAV-CMV-GFP injection. These results suggest T-cell infiltration of hypothalamus may be sufficient to cause obesity. Extrapolating the findings from these studies of human postmortem and injected mice, the results suggest as much as ~40% of human obesity may be driven by dysregulated T-cell responses in feeding circuits of the hypothalamus.

Abstract 2

Sam68 is a critical regulator of retinal angiogenesis

Corynn N Appolonia[1], Angela Rose Liu[1], Julia M Hluck[1], and Parameswaran Ramakrishnan[1,2,3,4]

[1]Department of Pathology, School of Medicine, Case Western Reserve University, Cleveland, OH, United States
[2]The Case Comprehensive Cancer Center, School of Medicine, Case Western Reserve University, Cleveland, OH, United States
[3]Department of Biochemistry, School of Medicine, Case Western Reserve University, Cleveland, OH, United States
[4]Louis Stokes Veterans Affairs Medical Center, Cleveland, OH, United States

Retinal angiogenesis refers to the formation of new blood vessels in the retina and occurs in both physiological and disease states. In proliferative diabetic retinopathy and wet age-related macular degeneration, pathological retinal angiogenesis occurs due to hypoxia and increased vascular endothelial growth factor (VEGF). Sam68 is an RNA-binding protein that is upregulated during hypoxia and regulates angiogenic sprouting in human umbilical vein endothelial cells. Its role in retinal angiogenesis is unknown. We tested the hypothesis that Sam68 is pro-angiogenic and required for retinal endothelial cell migration, cell sprouting, and lumen formation, which are key steps in angiogenesis. Sam68-knockout (KO) murine retinal endothelial cells (mRECs) were generated using CRISPR/Cas9 gene editing and were used to study angiogenesis and response to hypoxia. We discovered that Sam68-KO mRECs have significantly reduced cell migration, sprouting, and tube formation, suggesting deficits in angiogenic potential. In response to hypoxia, Sam68 protein levels increased in mRECs in a time-dependent manner. Compared to WT mRECs, Sam68-KO mRECs have significantly decreased VEGF gene expression in hypoxic conditions, which corresponds with a lack of increase in hypoxia-induced factor 1 alpha (HIF1α) protein levels. Our results validate our hypothesis that Sam68 is required for angiogenesis in retinal endothelial cells and indicate that Sam68 may regulate HIF1α protein expression during hypoxia. Our study is the first to investigate the role of Sam68 in retinal angiogenesis and suggests that inhibition of Sam68 may be a therapeutic strategy to reduce pathological retinal angiogenesis. We plan to use Sam68 inhibitors and an oxygen-induced retinopathy model in Sam68-KO mice to further investigate the therapeutic implications of our findings.

Abstract 3

Assessing milk transmission of bovine-origin H5N1 avian influenza virus in the lactating ferret model

Pari H. Baker*[1], Michelle Moyer*[1], Carolyn Lee[2], Yixuan Bai[1], Lauren S. Stafford[1], Scott P. Kenney[2], Stephanie N. Langel[1]

[1]Center for Global Health and Diseases, Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH, USA
[2]Center for Food Animal Health, Department of Animal Sciences, The Ohio State University, Wooster, OH USA

The current H5N1 avian influenza virus circulating in U.S. dairy cattle exhibits a distinct tropism for the mammary gland, with infected milk containing high levels of viral RNA and infectious virus. The virus's ability to infect the mammary gland raises concerns about transmission to offspring through breastfeeding. We utilized a lactating ferret model to study H5N1-induced mastitis and viral transmission in the lactating mammary gland. Lactating ferrets (n=2) were intramammary inoculated with 10^4 EID_{50}/gland of bovine-origin H5N1. Viral RNA in milk significantly increased over time and remained elevated in mammary gland tissue. All animals exhibited weight loss, reaching a humane endpoint threshold of 20% by 6-days post-infection (DPI). To assess milk-mediated transmission of H5N1 between lactating dams and neonates, a cross-fostering approach was employed. Ferret dams were intramammary inoculated with either saline or a high dose of H5N1 virus as previously described. At 2 DPI, kits from saline-inoculated dams were cross-fostered onto high-dose H5N1-infected dams, and vice versa. Kits born to H5N1-infected dams and fostered by saline control dams ultimately succumbed within 2 days post cross-foster. Conversely, kits born to saline-inoculated dams and fostered by H5N1-infected dams acquired the virus via milk and were euthanized on 4 DPI due to nearing a humane endpoint cutoff. These data demonstrate that H5N1 virus is transmitted through milk, resulting in 100% mortality among both dams and neonates. This model will be utilized to study H5N1 virus pathogenesis within the mother-infant dyad and to evaluate potential therapeutics for combating H5N1 virus infection and disease.

Abstract 4

*TNFRSF13*B polymorphisms impact antibody effector properties determining allograft immunopathogenesis

Mayara Garcia de Mattos Barbosa[1], Aghiad Daboul[1], Lwar Naing[1], Nancy Nagy[1], Neil Greenspan[1], Jeffrey L. Platt[1] and Marilia Cascalho[1]

[1]Pathology, Case Western Reserve University, Cleveland, OH44106

TNF receptor superfamily member 13B (*TNFRSF13B*) influences B plasma cell development after immunization. A high frequency of *TNFRSF13B* alleles encoding low-function receptors is surprising given association with common variable immunodeficiency. Exploring what might fuel polymorphism, we found that *Tnfrsf13b*-KO and low-function variant mice had decreased antibodies but were resistant to enteric pathogens, suggesting balancing selection. To explore mechanisms explaining the combination immunodeficiency and host resistance we examined the impact of low function *TNFRSF13B* variants in organ transplantation. We found that 21% of 177 recipients of kidney transplants with antibody-mediated rejection (AMR) but only 6% of 125 subjects with stable kidney function had low function *TNFRSF13B* alleles. Semi-allogeneic kidney transplants in *Tnfrsf13b*-mutant mice developed severe AMR with deposits of IgG and C3d, and decreased C3 in serum compared to milder graft damage in WT recipients. We found that mutant mice produce less IgM at baseline and after alloimmunization; IgM is especially effective at activating complement. Mutant mice produced more alloreactive IgG2b and IgG3, which effectively activate complement, than wild-type mice. Mutant mice had 2-fold less sialylated IgG Fc than WT mice. Decreased IgG Fc sialylation increased C1q binding explaining proinflammatory responses in grafts in *Tnfrsf13b*-mutant hosts. The findings are consistent with the proposition that *TNFRSF13B* polymorphism and low functioning variants are maintained by balancing selection. Low-function variants confer increased immunity to infection and to allogeneic cells/organs but at a biological cost that includes increased prevalence of primary immunodeficiency.

Abstract 5

HCMV Restricts the Innate Immune Response by Nuclear Export of Host Restriction Factor DDX41

Vargab Baruah[1] and Christine M. O'Connor[1]

[1]Infection Biology; Sheikha Fatima bint Mubarak Global Center for Pathogen and Human Health Research, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, 44195 USA

Human cytomegalovirus (HCMV) is a ubiquitous dsDNA beta-herpesvirus with no vaccine and limited treatment options. While primary HCMV infections are typically asymptomatic in immunocompetent individuals, they can cause serious disease in those with naïve or compromised immune systems. Due to the importance of immune status in establishing infection and the role of innate immunity as the first line of defense, HCMV has evolved multiple mechanisms to manipulate host interferon (IFN) responses. DEAD-Box Helicase 4 (DDX41) is an intracellular dsDNA sensor that depends on the Stimulator of Interferon Genes (STING) signaling pathway to trigger type I IFN production. We found that DDX41/STING signaling is upregulated and activated during lytic HCMV infection. Both DDX41 and STING, along with the DDX41-activating kinase, Bruton's tyrosine kinase (BTK), are activated through tyrosine phosphorylation. Furthermore, we observed that DDX41 and BTK physically interact with STING during lytic infection. Pharmacological intervention with the BTK inhibitor orelabrutinib attenuated DDX41 phosphorylation/activation, leading to increased expression of viral proteins and virus replication. Importantly, HCMV infection re-localizes DDX41 from the nucleus to the perinuclear virus assembly compartment, excluding DDX41 from the viral DNA replication sites in the host cell's nucleus. This cytoplasmic DDX41 interacts with HCMV tegument protein pp65 and exhibits attenuated phosphorylation, suggesting that its HCMV-induced redistribution renders DDX41 less active or inactive. In sum, our work reveals that DDX41/STING signaling is important for the innate immune response against HCMV, and that HCMV subverts this response by displacing DDX41 from the nucleus, diverting it from its protective role.

Abstract 6

Long-acting IL-7 Induces Lymphoid Aggregates in the Tumor Microenvironment

Dijana Bjelivuk[1], Dathan Andrews[1], Namita Nanda[1], and Rebecca Obeng[1-3]

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[2]Department of Pathology, University Hospitals Cleveland Medical Center
[3]Case Comprehensive Cancer Center, Case Western Reserve University School of Medicine

The cytokine, Interleukin-7 (IL-7), plays an important role in the immune system by supporting B and T cell development. It also supports T cell survival and memory differentiation. More importantly, it has been shown to promote the formation of tertiary lymphoid structures (TLSs). TLSs are associated with enhanced clinical response to immunotherapy and overall survival of patients with cancer. Therefore, treatment with IL-7 could improve responses to cancer immunotherapy through the creation of TLSs. However, IL-7 has a short half-life that limits its use in patients. In this study, we evaluated the ability of a long-acting form of IL-7 to induce TLSs in the tumor microenvironment. Lewis lung cancer (LLC) cells were seeded in the lungs of mice by tail vein injection. After 7 days, mice were treated with two doses (200 µg/mouse) of the long-acting IL-7, 7 days apart. On day 22 after tumor inoculation, we harvested the tumor-bearing lungs and performed immunofluorescence staining to assess lymphoid aggregates and TLSs in the control and treated groups. We observed more aggregates in the IL-7 treated group compared to the control group. The aggregates within the treated groups were larger, and some were composed of tight clusters of T and B cells, suggestive of TLSs. Our data suggest that the long-acting IL-7 can induce lymphoid aggregates and potentially TLSs. Thus, long-acting IL-7 may provide therapeutic benefit to patients with cancer.

Abstract 7

The oral microbiome is dynamic during CAR T-cell therapy

Emily Blaum[1,2], Naseer Sangwan[3,4], Aaron Miller[4], Manishkumar S. Patel[1], Mark Brown[5], Brian T. Hill[6], and Neetu Gupta[1]

[1] Department of Inflammation and Immunity, Lerner Research Institute, Cleveland Clinic; [2] Medical Scientist Training Program, Case Western Reserve University; [3] Microbial Sequencing and Analytics Core, Lerner Research Institute, Cleveland Clinic; [4] Department of Cardiovascular and Metabolic Sciences, Lerner Research Institute, Cleveland Clinic; [5] Department of Cancer Biology, Lerner Research Institute, Cleveland Clinic; [6]Hematology and Medical Oncology, Taussig Cancer Institute, Cleveland Clinic

Anti-CD19 chimeric antigen receptor (CAR) T-cell therapy has been a breakthrough for patients with hematologic malignancies who have not responded to traditional therapy. However, most patients will experience disease relapse and acute toxicities. The environment of the patient during therapy is complex; to best predict patient response and toxicity to therapy, biomarker studies must evaluate multiple, diverse sources. Here, we assessed the ability of the oral microbiome to serve as a novel biomarker and aimed to identify if there were distinct microbial compositions that were associated with clinical milestones to CD19-directed CAR T-cell therapy. To address these questions, we collected saliva samples at four time points during therapy (day of apheresis, day of CAR T-cell infusion, and days 7 and 14 post-infusion) from 80 patients with relapsed/refractory lymphoma. In a pilot study, microbiome profiling was performed with 16S rRNA sequencing using samples from eight patients. By determining the relative abundance of microbial genera, we demonstrate that specific microbes peak in abundance at distinct time points, allowing us to identify microbial biomarkers of clinical milestones. Furthermore, beta diversity revealed samples grouped by day of therapy, not by patient, suggesting the oral microbiome is distinct at different clinical time points. This pilot study demonstrates the feasibility of using saliva samples to assess the state of the microbiome in CAR T-cell patients and highlights its dynamic nature during therapy, supporting the further analysis of our cohort to identify microbial biomarkers of response, survival, and acute toxicities.

Abstract 8

Macrophage Mechanosensor Transient Receptor Potential Vanilloid 4 (TRPV4) and NF-κB Interact Inhibiting Bacterial Pneumonia-Induced Lung Injury

A. Boulton[2], M. Grund[2], Y. Wang[2], S. Abraham[2], L.M. Grove[2], B.D. Southern[1,2], Amanda Reinhardt[2], Erica M. Orsini[1], M. A. Olman[1,2], R. G. Scheraga[1,2]

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RATIONALE: Pulmonary macrophages are crucial for clearing bacterial infections but can induce lung tissue injury through pro-inflammatory cytokine secretion, leading to acute respiratory distress syndrome (ARDS). We have previously reported that the macrophage inflammatory response to pathogens depends on a soluble and mechanical signal. We undertook this study to define the intracellular mechanism whereby the mechanosensitive cation channel, Transient receptor potential vanilloid 4 (TRPV4), regulates the macrophage pro-inflammatory response to pathogens.

RESULTS: The lung injury response was assessed by inflammatory cell infiltration and intracellular cytokine production in *Trpv4*$^{fl/fl}$ and Cd11c-specific *Trpv4* KO mice (*Trpv4*$^{Cd11c\text{-}cre}$) after *Pseudomonas aeruginosa* infection. *Trpv4*^{Cd11cMcre} mice showed increased lung injury and immune cell infiltration compared with *Trpv4*$^{fl/fl}$. *Trpv4 KO* BMDMs revealed an increase in pro-inflammatory gene expression with TLR1/2 and 4 agonism, suggesting broad TRPV4-dependent inhibition of the NF-κB pathway. Conversely, overexpression of TRPV4 suppressed NF-κB promoter transcriptional activity in a luciferase reporter assay. Loss of function (pharmacologic or genetic deletion) of TRPV4 enhanced IL-1β secretion in murine and human ARDS-derived macrophages, as measured by ELISA. This secretion was blocked by knockdown of the NF-κB pathway. *In silico* modeling implicates TRPV4's ankyrin repeat domain (ARD) as the site of NF-κB interaction. NanoBiT experiments confirm that TRPV4's ARD is critical for its binding to NF-κB/p65, with TRPV4$^{\Delta Ard}$ exhibiting a significant loss of interaction *in vitro*.

CONCLUSIONS: Collectively, these data point to a critical interaction between TRPV4 and p65 that regulates NF-κB signaling and thereby identifying a novel target for therapeutics in ARDS.

Abstract 9

Nuclear estrogen receptors modulate CD4$^+$ T cell responsiveness to TGFβ

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TGFβ signaling is required for numerous T cell functions, including differentiation of naïve T cells into regulatory T cells (Tregs) and Th17 cells. Previous studies have shown that 17β-estradiol (estrogen, E2) signaling through its nuclear receptors alpha and beta (ERα and ERβ) can modulate TGFβ signaling in epithelial cancer cell lines. Additionally, our lab has demonstrated that enhanced ERα-specific signaling (in murine naïve CD4$^+$ T cells lacking ERβ) inhibits TGFβ-dependent Foxp3 expression, further suggesting a relationship between E2 and TGFβ signaling pathways. This led us to hypothesize that E2 signaling impacts the responsiveness of CD4$^+$ T cells to TGFβ. To test this, we isolated CD4$^+$ T cells from the spleens and mesenteric lymph nodes of female wild-type (WT) mice or mice harboring global deletions of ERα or ERβ (ERα-KO, ERβ-KO) and treated these cells with E2 and TGFβ. We found that E2 pre-treatment reduces TGFβ-induced phosphorylation of Smad3, indicating reduced activation of TGFβ signaling. To investigate the effects of E2/TGFβ crosstalk on the T cell transcriptome, we conducted a bulk RNA-sequencing analysis on CD4$^+$ T cells treated with αCD3/CD28, E2, and TGFβ. This analysis revealed differential expression profiles of genes associated with TGFβ signaling, with E2-treated cells showing elevated expression of TGFβ-signaling regulators. We further investigated the responsiveness of naïve CD4$^+$ T cells to TGFβ via *ex vivo* Treg differentiation assays and characterized expression of Treg-specific markers. Our results suggest that ERα- versus ERβ-dominated signaling could alter phenotypes of differentiated Tregs, which could be a result of altered TGFβ responsiveness.

Abstract 10

Inflammatory signaling in endothelial cells drives changes in IgG sialylation

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A core component of the humoral immune response is IgG, an antibody class distinguishable by its unique heavy chain composition and by its integral role in protective immunity as well as immunologic homeostasis. Composition of the N-glycan at the conserved N297 site within the Fc domain is a critical point of regulation that influences the function of IgG through alteration in Fc domain conformation. Terminal α2,6 sialylation of this glycan by the sialyltransferase ST6Gal1 has been shown to contribute to a homeostatic immune state through suppression of receptor signaling. Epidemiologic data over the past four decades has further demonstrated that inflammation is linked to a loss of both sialic acid and galactose residues on IgG, presumably to enhance the pro-inflammatory environment. In our previous and recent work, we have discovered that the endothelium rather than B cells is the dominant location at which IgG is sialylated. However, understanding the regulation of ST6Gal1 and thereby IgG sialylation in endothelial cells (ECs) remains a significant knowledge gap that poses a hurdle in obtaining a broadened understanding of IgG functional regulation. Here, we report that ECs drastically downregulate ST6Gal1 expression in response to inflammatory stimuli such as IFNγ. This response appears to be different than B cells, possibly due to EC exclusive use of the ST6Gal1 P3 promoter. Moreover, inflammatory stimuli also decreased the ability of ECs to sialylate IgG. These data support a model in which endothelium-specific inflammatory regulation of ST6Gal1 drives the extent of plasma IgG sialylation.

Abstract 11

WNK1 is a Novel Regulator of Aberrant Signaling in Acute Myeloid Leukemia

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Acute Myeloid Leukemia (AML) is the most common acute leukemia in adults. The overall survival rate is relatively poor (around 30%) compared to other hematological malignancies. This is primarily due to higher rates of relapse and chemoresistance. In the effort to overcome the suboptimal outcomes of chemotherapy, recent research efforts have focused on targeting molecular abnormalities in AML patients. In addition to providing chemotherapy-free treatment options, molecular-based therapies are promising because they have the potential to disrupt the development of key disease features such as hyperproliferation, apoptosis resistance, and differentiation arrest. Kinases are key mediators of cellular signaling pathways and aberrant kinase activation has been observed AML. Mutations in tyrosine kinases, such as FLT3, cause constitutive activity which leads to downstream oncogenic signals. Tyrosine kinase inhibitors have shown promise in early phase clinical trials. However, they have displayed limited success in patients lacking tyrosine kinase mutations. It is imperative that we discover additional kinases that contribute to AML progression that could offer additional targets for AML treatment. We identified With-no-Lysine(K) kinase 1 (WNK1) as a dysregulated kinase in AML that could be contributing to disease pathogenesis as we observed that AML patients had elevated WNK1 expression and that higher WNK1 expression correlated with worse overall survival. We found that inhibiting WNK1 kinase function resulted in decreased viability and cell cycle progression of AML cells. In addition, inhibiting the well-established substrates of WNK1: Ste20-related proline-alanine-rich kinase (SPAK) and Oxidative stress responsive kinase 1 (OSR1), caused similar effects. Inhibiting WNK1 also induced AML cell differentiation which was further enhanced by combination treatment with ATRA. Taken together, our findings implicate a role for WNK1 signaling in promoting AML survival, growth, and dedifferentiation.

Abstract 12

High-throughput compound screening reveals small molecule mediators of NK cytotoxicity against solid cancers

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Adoptive cell therapies (ACT), such as CAR-T and NK therapy, have transformed hematologic cancer treatment but show limited efficacy against solid tumors. NK cell therapies in particular have an excellent safety profile due to their specific, innate recognition of cancer cells, but fail to elicit adequate and sustained tumor clearance *in vivo*. To address this hurdle, we developed a high-throughput phenotypic small molecule screen to identify novel compounds that can enhance NK cell cytotoxicity against tumor cells.

We screened 150,000 compounds for their ability to enhance NK cytotoxicity against ovarian cancer cells (OVCAR3). Our primary screen and subsequent hit validation identified 10 lead compounds that increase NK cell killing of OVCAR3 cells (>10% increase over vehicle-treated control). We performed secondary screening using a flow cytometry-based assay and confirmed that 5 compounds significantly enhance NK cytotoxicity against OVCAR3. One lead compound in particular - NKSM01 – has been further shown to increase NK cytotoxicity after compound washout, as well as increase IFNγ secretion by NK cells co-cultured with OVCAR3. The next steps in our study include evaluating *in vivo* efficacy of NKSM01 and other hit compounds, as well as exploring cellular mechanisms by which lead compounds potentiate NK cytotoxicity. Our findings will contribute to future clinical trials and NK cell therapies with greater efficacy against solid cancers.

Abstract 13

Oncostatin-M Ligand-Based CAR-T Therapy Targets and Eliminates Osteosarcoma

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Osteosarcoma is the most common neoplasm of the bone in children and adolescents, accounting for 5% of all childhood cancers. While surgical advances and chemotherapy have improved patient outcomes, no new therapies have been approved in over 30 years. Oncostatin-M (OSM) signaling through oncostatin-M receptor (OSMR) has been shown to be critical for cancer progression and metastasis, including in osteosarcomas. OSMR is expressed on the surface of both cancer associated fibroblasts and cancer cells themselves. To target OSM receptors we generated OSM-ligand-expressing CAR-T cells. Importantly, these OSM CAR-T cells demonstrate impressive killing against immortalized osteosarcoma cell lines, but not cells lacking OSM receptors. Additional in vitro testing against patient derived cells and xenografts showed elimination of cancer cells. Moreover, just one injection of OSM CAR-T cells significantly reduced tumor burden in vivo against immortalized cell lines and an aggressive newly-characterized osteosarcoma PDX model. OSM CAR-T cells also reduced metastasis in mouse lungs and livers, but did not show significant cytotoxicity against normal human bronchial epithelial cells or hepatocytes. Cytokine analysis of serum showed large releases of granzymes, perforin, and IFN-gamma only in CAR treated mice. A mouse safety study showed no signs of toxicity post OSM CAR-T injection for up to 6 months. Whole mouse CryoViz imaging showed that intravenously injected CAR-T cells did not directly home to off target locations. Taken together, this study shows a potential new CAR-T cell target, characterizes a new PDX model, and demonstrates a potential ligand-based CAR-T therapy for osteosarcoma.

Abstract 14

A Strategy to Generate Anticipatory Immunity Against Mutable Viruses

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HIV infection and transmission remain a global health concern, and no vaccine strategy has succeeded in preventing HIV transmission and infection. HIV, like many other RNA viruses, lacks proofreading mechanisms and diversifies faster than host immunity can develop. HIV primarily infects and develops latency within CD4+ lymphocytes and macrophages. Previous HIV vaccine trials, HVTN505 and RV144, have utilized this fact to stimulate T cells using a vector-based prime-boosting strategy. However, they failed to prevent broad immunity because immune responses only develop against a subset of the viral antigens encoded by HIV variants. Instead, we propose testing a novel strategy, which we call the “mutable vaccine.” The mutable vaccine concept hypothesizes that diversification of viral antigens by co-opting the somatic hypermutation mechanism in B cells followed by secretion of viral variants will generate broadly reactive immunity that will curtail viral evolution in vaccinated individuals. To test the hypothesis, we have established a murine model of the mutable vaccine by introducing an HIV-1B Env transgene under the control of the murine Ig lambda light chain promoter and murine Ig heavy chain intronic enhancer, conditionally expressed and mutated following activation-induced cytidine deaminase (AID) expression. We show that HIV Env mutates as often as the Ig VH exons. Furthermore, $CD8^+$ T cell depletion resulted in higher frequencies of Env mutations within B cells. Prominent mutations enhanced MHC-1 binding affinity, suggesting that this strategy may induce cell-specific immunity against B cells and effectively eliminate viral reservoirs in infected individuals.

Abstract 15

Transient Receptor Potential Vanilloid 4 (TRPV4) increases *Burkholderia cenocepacia* bacteria clearance in macrophages *in vitro* and lung infection *in vivo* via regulation of autophagy

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Burkholderia cenocepacia is an intracellular pathogen that causes severe pneumonia in cystic fibrosis (CF) patients, evading immune responses and macrophage-mediated clearance. Autophagy, a key mechanism for intracellular pathogen clearance, plays a critical role in combating *B. cenocepacia*. We previously showed that the Transient Receptor Potential Vanilloid 4 (TRPV4) channel in macrophages is essential for clearing extracellular pathogen *Pseudomonas aeruginosa*. However, its role in clearing *B. cenocepacia* has not been explored. Here, we investigate the role of TRPV4 in autophagy to control clearance of *B. cenocepacia* in macrophages *in vitro* and *in vivo*.

In vivo, *Trpv4*-/- mice exhibited higher bacterial burdens in the lungs after *B. cenocepacia* infection compared to wild-type mice, indicating impaired bacterial clearance. *In vitro*, TRPV4-deficient bone marrow-derived macrophages (BMDMs) showed increased intracellular *B. cenocepacia* and defective autophagosome formation, evidenced by decreased autophagy-related proteins (Beclin1, ATG12-ATG5, ATG16L) and autophagosome markers (LC3BI/II), as well as elevated p62 levels, indicating impaired degradation of bacterial cargo. Autophagy flux reporter macrophages confirmed TRPV4 downregulation inhibited autophagy.

These findings suggest that TRPV4 is crucial for autophagy-mediated clearance of *B. cenocepacia* in macrophages. Targeting TRPV4 may enhance bacterial clearance in pneumonia, offering a potential therapeutic strategy.

Abstract 16

Agent-based modeling of parasite-immune malaria system with applications to data analysis, disease intervention, and evolution of drug resistance

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The talk will outline an agent-based model of multi-clone Pf malaria with a detailed account of within-host biology, and mosquito transmission. On the host side, it features parasite genetic makeup (VSA), its immune evasion strategies (antigenic variation), stimulation of specific immune effectors, parasite clearing, and RBC depletion. Different parasite clones can recombine in mosquito, generating multi-clonal diversity, which contributes to evolution of adaptive parasite traits, virulence, persistence, drug resistance on population level.

We shall demonstrate model application to malaria-therapy dataset, the effect of chemotherapy in seasonal transmission environment, the onset of drug resistance.

Abstract 17

Insights into heterologous immunity: ELISpot analysis of diverse mouse stimulator-responder strain combinations

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In clinical transplantation, ELISpot assays have been used to measure the pre-transplant frequency of donor-reactive heterologous memory T cells, which correlates with immune-mediated graft injury and function. In murine transplant models, ELISpot assays can likewise be used pre-transplant to measure the frequency of donor- reactive heterologous memory T cells. A prototypic murine solid-organ transplant model is complete MHC mismatched A/J (H-2^a) grafts to C57BL/6 (H-2^b) recipients. However, a systematic characterization of mouse strain combinations to determine which mouse strains respond strongest and weakest to allogeneic transplant after co-culture has not been performed, an important consideration for murine transplantation research. Here we systematically characterized the frequency of heterologous donor-reactive memory T cells in unsensitized mouse splenocytes. Using the ELISpot assay, we measured the frequency of pre-existing donor-reactive heterologous memory CD8 T cells as a function of IFNγ and granzyme B production following 48 hours of culture of splenocytes from unsensitized C57BL/6, A/J, and BALB/c (H-2^d) mice in the presence or absence of supplemental IL-15 – a cytokine that exposes granzyme B-producing heterologous memory T cells. We identified C57BL/6 mice as robustly responding to a variety of allogeneic stimuli. Despite being potent allogeneic stimulators, A/J and BALB/c mice are poor responders to intercross allogeneic stimulation as well as by C57BL/6 stimulator cells. This systematic characterization of murine responses to allogeneic stimulation can be used to guide future basic and translational transplant research by informing researchers on which mouse strain pairs have the strongest and weakest heterologous immune responses to allogeneic stimulation.

Abstract 18

CRISPR-Based Kinome Screening to identify new kinase targets for lung cancer treatment

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Lung cancer is a leading cause of cancer-related mortality globally and resistance to its treatment is a critical barrier to improved outcomes. We studied a kinome-wide CRISPR knockout screening approach aimed to systematically identify kinases that are important for tumor survival and therapy resistance *in vivo*. We used a murine Lewis lung carcinoma model and subcutaneously implanted CRISPR-edited LLC cells to monitor the effects of kinase knockout in control versus treatment group. Next generation sequencing results from two independent screens revealed distinct patterns of genetic enrichment and depletion, with specific kinase targets consistently overrepresented or underrepresented in resistant tumors. For preliminary studies, LLC and MC38 cell lines were treated with two CDK-12 inhibitors and the results demonstrated potent dose-dependent cytotoxicity, with one compound achieving near-complete tumor cell death at low nanomolar concentrations. Additionally, altered expression of PD-1—a key regulator of antitumor immunity—suggested interplay between kinase signaling and immune evasion mechanisms. These findings highlight dual therapeutic opportunities that involves direct targeting cancer cell survival mechanisms and disrupting resistance-associated immunosuppressive pathways. The identification of kinases with tumor-intrinsic and immune microenvironmental factors provides a rationale for developing combination therapies that integrate kinase inhibition with immunomodulatory strategies. Further mechanistic studies are needed to elucidate relationships between kinase activity and immune response dynamics.

Abstract 19

Carbohydrate malabsorption as a potential mechanism underlying selective IgA deficiency

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IgA is a critical immune mediator capable of neutralizing pathogens and their toxins within the intestinal lumen. The importance of IgA is highlighted by the complications associated with selective IgA deficiency. Side effects of pharmaceuticals are the most common non-genetic cause of selective IgA deficiency with antibiotics often implicated in its development. I found that gavage of mice with vancomycin resulted in a rapid fall in fecal IgA levels within 48 hours of treatment. By isolating luminal contents from each of region the intestine, I determined that luminal IgA concentrations were drastically diminished in the lower intestine beginning in the cecum following vancomycin gavage. Investigation of immune and epithelial cells along each segment of the intestine did not reveal any alterations in IgA production or transport. Instead, decreased luminal IgA concentrations were accompanied by a reciprocal increase in water levels. Because carbohydrates exert a strong osmotic force relative to other macronutrients, I investigated the role of dietary carbohydrate intake in mediating this phenotype. I found that luminal water levels and dietary carbohydrate intake positively correlate but only in the context of vancomycin treatment, unmasking the role of vancomycin-sensitive microbes in the maintenance of proper water balance in the lower intestine. Based on these data, I believe there is compelling evidence linking the loss of carbohydrate catabolism by the microbiota to decreased fecal IgA levels following vancomycin gavage. Further investigation of this phenomenon will improve understanding of how antibiotics influence mucosal immunity and potentially uncover previously unappreciated mechanisms of immunodeficiency.

Abstract 20

Osteosarcoma Utilizes VCAM1-VLA4 Signaling Axis on Macrophages to Promote Pulmonary Metastasis

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Osteosarcoma (OS) is an aggressive bone cancer primarily found in children and young adults with a five-year survival rate that drops significantly in patients with metastatic disease. At diagnosis, around 20% of patients have lung metastases, reducing survival from 70% to 30%. We believe that an indicator of metastatic potential is the aberrant expression of Vascular Cell Adhesion Molecule 1 (VCAM-1) on the tumor surface as human OS tissues have been shown to overexpress this molecule; however, its precise role remains unclear. We hypothesize that the interaction between VCAM-1 on OS cells and its receptor, the α4β1 integrin (VLA-4) on macrophages, contributes to the ability of OS to metastasize. Our preliminary evidence suggests that tumorigenic effects of VCAM-1 are isoform dependent, and truncated VCAM-1 is critically important to metastasis. Our research model uses bone marrow-derived macrophages exposed to murine OS cell lines K7 and K7M2, the latter being highly metastatic. Early data show that VCAM-1-VLA-4 binding induces a pro-tumoral macrophage phenotype, upregulating Arginase 1, an M2 macrophage marker. Currently, we are investigating whether this effect is specifically driven by the truncated isoform and exploring the role it plays in the PI3K-AKT signaling pathway. We plan to evaluate this hypothesis by looking at expression of the proteins in this signaling axis on bone marrow derived macrophages (BMDMs) that have been influenced by the aforementioned osteosarcoma cell lines. By elucidating how VCAM-1-VLA-4 interactions drive macrophage polarization, we aim to identify novel therapeutic targets that could shift macrophage behavior and improve treatment outcomes in OS.

Abstract 21

Investigating the regulation and functions of PRSS1 and PRSS2 in natural killer cells

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Natural killer (NK) cells and $CD8^+$ T cells are two types of cytotoxic lymphocytes, which are the body's primary effectors against virally infected and cancer cells. Both rely on the release of cytolytic granules containing various hydrolytic enzymes to achieve target cell elimination. Despite this understanding, the contents of cytolytic granules have yet to be fully characterized and the scope by which proteases mediate cytotoxicity requires further investigation. Using RNA sequencing to identify NF-κB-regulated proteases in NK cells, we found that *Prss1* and *Prss2* are expressed in murine NK cells, and that their expression is significantly decreased in NK cells lacking the NF-κB subunit c-Rel. Our preliminary data further demonstrates that *PRSS1* and *PRSS2* are expressed in human NK cells. Surprisingly, we observed very low levels of *PRSS1* and *PRSS2* in human $CD8^+$ T cells relative to NK cells. Lastly, our preliminary data shows that murine NK cells lacking PRSS2 exhibit decreased cytotoxicity. We hypothesize that PRSS1 and PRSS2 are a part of the uncharacterized subset of enzymes present in NK cell cytolytic granules and that they play key roles in NK cell cytotoxicity. Future directions will include validating the localization of PRSS1 and PRSS2 in NK cells, elucidating the mechanisms regulating their expression, and further exploring their impact on NK cell effector functions. Ultimately, uncovering the roles of PRSS1 and PRSS2 in NK cells may lead to the identification and targeting of novel cytotoxic mechanisms that increase the therapeutic efficacy of NK cell-based immunotherapies.

Abstract 22

Mast Cell Derived Histamine Negatively Regulates Hematopoiesis

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Hematopoietic stem cells (HSCs) play a crucial role in generating all blood cell types, vital for a functional immune system and oxygen transport. Maintaining this balance throughout life involves a tight regulation of self-renewal, differentiation, and quiescence, influenced by both intrinsic and extrinsic signals. While the influence of many HSC progeny on HSC decisions is known, the role of mast cells (MCs) has remained unexplored. MCs, known for their immunomodulatory functions through secretion of various factors including histamine, present a novel avenue for understanding HSC regulation. In this study, we uncover a novel role for MC-derived histamine in modulating HSC behavior. Our hypothesis posits that MCs act as negative regulators of HSCs. We observed that genetically MC-deficient “SASH” mice exhibit increased hematopoietic output and bone marrow (BM) HSCs, characterized by an enhanced quiescent signature that increases chemoresistance. The SASH microenvironment also shows elevated frequencies of HSC-supportive cell types and increased expression of genes conducive to HSC maintenance, providing a functional advantage when wild-type BM is transplanted into this microenvironment. Moreover, we found that the genetic loss of MCs correlates with lower serum histamine levels in SASH mice, and the augmented hematopoietic phenotype can be reversed by administering exogenous histamine. Subsequent experiments with FDA-approved antihistamines in wild-type mice revealed that cetirizine, an H1R inverse agonist, notably increased HSC frequency in the BM. Overall, our findings highlight MCs as negative regulators of HSCs, laying the groundwork for future studies to unravel the underlying mechanisms and explore the therapeutic potential of cetirizine.

Abstract 23

NK cell mediated allorecognition shapes reprogramming of neutrophils within heart allografts

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Recent developments in neutrophil biology have demonstrated that neutrophils are phenotypically and functionally heterogeneous. Tissue microenvironments dictate changes in neutrophil cell states in infection and cancer, but little is known about how alloimmune responses or solid organ transplantation influence neutrophil heterogeneity and plasticity. Here, using the murine heterotopic heart transplant model, we identified substantial skewing of early graft infiltrating neutrophil populations upon entering a heart graft towards a mature, aged, and pro-angiogenic dcTRAIL-R1 expressing phenotype. Further, we uncovered striking differences between neutrophils infiltrating complete MHC mismatched allografts and syngeneic isografts. Early after transplant in both allograft and isograft recipients, bone marrow neutrophil development was highly skewed towards an immature, interferon stimulated gene (ISG)+ subset, which was also the dominant population in the peripheral blood. In contrast, neutrophils maintained the ISG+ phenotype after entering an allograft but appeared to turn off this program upon entering an isograft. Interestingly, while the existing literature indicates that IFIT1-expressing ISG+ neutrophils, and proangiogenic dcTRAIL-R1+ neutrophils are distinct subsets, we identified a novel IFIT1+ dcTRAIL-R1+ hybrid population that is highly enriched in allografts. Mechanistically, we show that NK cell-mediated innate allorecognition drives this early allograft specific neutrophil phenotypic programing. These findings provide novel insights into the regulation of recently described key neutrophil subsets that could enable more specific targeting to neutralize detrimental neutrophil subsets and enhance solid organ transplant outcomes.

Abstract 24

Loss of CD22 affects macrophage derivation and function

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Macrophages are highly plastic cells of the innate immune system capable of shifting their phenotypes and functions in response to various environmental cues. Our lab has demonstrated the importance of the sialic acid-binding immunoglobulin-like lectin (Siglec) CD22, previously thought to be expressed exclusively in B cells, in the context of the M2-like, immunomodulatory phenotype of macrophages. We have recently discovered that CD22 assists in the immunologically silent clearance of sialylated proteins and apoptotic cells without robust stimulation of T cells through its recognition of α2,6-linked sialic acids on this debris. In the present study, we expand our work regarding CD22 function in macrophages to include a knockout model of CD22 (CD22KO). Through RNA sequencing analysis, we have demonstrated that loss of CD22 affects baseline macrophage derivation, with unstimulated CD22KO bone marrow-derived macrophages (BMDMs) exhibiting transcriptional differences in comparison to WT BMDMs. While loss of CD22 does not impact the expression of canonical markers of polarization, such as CD206 and CD301 for the M2-like subset and CD86 for the M1-like subset, polarized CD22KO cells are transcriptionally distinct from polarized WT cells. Further, the function of M2-polarized CD22KO macrophages closely resembles that of M2-polarized WT macrophages lacking CD22 expression (CD22-). Ongoing work in our laboratory focuses on assessing the impact of CD22 abrogation in macrophage derivation, understanding the distinct and dynamic transcriptomes of CD22KO cells in response to polarization stimuli, and further exploring the functional consequences of the loss of CD22KO in macrophage subsets.

Abstract 25

Unconventional Myosin 18A is a novel regulator of the intestinal epithelial barrier and mucosal inflammation

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Establishment of the intestinal epithelial barrier is critical for the healthy gut homeostasis, while disruption of this barrier is known to ignite and exaggerate mucosal inflammation. Gut barrier permeability is regulated by the assembly of epithelial intercellular junctions and the coupling to underlying actin filaments enriched with the non-muscle myosin II (NMII) motors. In addition to NMII, intestinal epithelial cells (IEC) express several unconventional myosins, which roles in regulating epithelial junctions are poorly understood. Unconventional myosin 18A (Myo18A) is a unique multifunctional scaffold protein lacking myosin motor activity but possessing a PDZ binding domain, with the potential to interact with different junctional proteins. Therefore, we investigated the roles of Myo18A in regulating gut barrier permeability, epithelial junction integrity and mucosal inflammation.

Myo18A colocalized with epithelial apical junctions in healthy murine and human colonic mucosa. The junctional localization and protein expression of Myo18A was markedly decreased in the inflamed epithelium from ulcerative colitis patient samples. CRISPR/Cas9 mediated knock-out of Myo18A in human IEC disrupted the epithelial barrier and increasing perijunctional actomyosin contractility, leading to cell morphology changes. The increased actomyosin contractility in Myo18A-deficient cells was associated with enhanced phosphorylation of myosin light chains and recruitment of Rho-associated kinase to apical junctions. Consistently, mice with tamoxifen-inducible deletion of Myo18A in the intestinal epithelium showed increased gut permeability *in vivo*. Myo18A conditional knockout animals were protected from dextran sodium sulfate-induced colitis. Together, our findings revealed a novel cytoskeleton-dependent mechanism that regulates permeability of the intestinal epithelial barrier and epithelial responses to mucosal inflammation.

Abstract 26

The vaginal microbiome pre-vaccination associates with local and systemic HIV vaccine immune responses

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Background: In order to be effective, vaccination strategies against HIV should aim to induce mucosal immunity at HIV's primary acquisition site, the female genital tract. Previous studies have already demonstrated that the host microbiome can influence vaccine immunogenicity, but limited data exists on the role of the vaginal microbiome on vaccine-induced antibody response. Here we assess the relationship between the vaginal microbiome and vaccine-induced binding antibody (BAb) responses.

Methods: Cervicovaginal mucus samples were collected from participants (n=95) receiving the RV144 ALVAC-HIV/AIDSVAX B/E prime/boost vaccine regimen at baseline and 2 weeks post-RV144 prime/boost regimen. Vaginal microbiome data was generated by label-free tandem mass spectrometry. Mucosal and serum BAb levels were measured using ELISA, and differences between microbiome groups were determined by t -tests. Linear models were used to analyze combinations of dominant host microbes.

Results: A total of 24 unique genus- and species-level taxa were identified. Participants clustered into 3 microbiome groups: Lactobacillus (L.) crispatus dominant (n=20, 21.1%), L. iners dominant (n=40, 42.1%) and a polymicrobial group (PM, n=36.8, 36.8%). Relative to the L. crispatus group, women with a PM microbiome showed increased activation of pathways involved in B and T cell receptor signaling, MHC class I/II antigen presentation, and neutrophil degranulation (Z score>5; P<0.0001). A subset of women sampled had a consistent Lactobacillus dominant (LD, n=43) or non-Lactobacillus dominant (nLD, n=18) microbiome type across all examination timepoints. These sub-groups were similar in age (avg=29.6, avg=28.5, P=0.4). Consistent LD women showed significantly higher BAb levels compared to consistent nLD women at 2 weeks post-RV144 regimen, including those in the mucosa against A244 (IgG-gp120A244gD-D11, P=0.0393), and in serum (gp70V1V2-92TH023, P=0.004; IgG-A244, P=0.0153). Antibody gp70E also demonstrated significant enrichment in serum (IgGtogp70E, P=0.004). In linear models, dominant microbes related to *Lactobacillus* predicted BAb levels (A244 in serum p=0.18 and in mucus p= 0.0051, and gp70E in serum p=0.0092). T-tests and linear models examining relationships between the host proteome and antibody level identified over 100 proteins displaying significant associations across multiple analyses.

Conclusion: These data suggest an association between the vaginal microbiome and vaccine-induced BAb responses, with a more inflammatory polymicrobial environment being associated with lower vaccine-specific BAb levels. Preliminary analysis implies *Lactobacillus* microbes may either be promoting HIV vaccine response, or are a marker of HIV vaccine immunogenicity. These results warrant further interrogations of potential mechanisms by which the vaginal microbiome may impact vaccine responses and could inform interventional strategies.

Abstract 27

Differential Proinflammatory Cytokine Expression and the Immune Response to Bacterial Vaginosis: the THRIVE Study

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Bacterial vaginosis (BV), characterized by vaginal *Lactobacillus* depletion and replacement by diverse anaerobic bacteria, is associated with adverse reproductive health outcomes. However, the role of host immunity in pathogenesis and treatment remains unclear. The THRIVE study examined immune cells and soluble mediators in vaginal mucosa of Canadian women categorized clinically as BV+ or BV- according to Amsel criteria or Nugent scoring. Antibiotic treatment was provided per routine clinical care at enrollment. Samples were collected at baseline, 1-month, and 6-month timepoints. Cytobrushes were immunophenotyped by flow cytometry, cervicovaginal lavages were analyzed by label-free mass spectrometry and 16S rRNA sequencing, while cytokines were measured via Luminex. Molecular BV was defined as non-*Lactobacillus* dominant (nLD) 16S profiles where <50% of bacterial proteins annotated to *Lactobacillus*.

Of 52 participants, 19 were diagnosed BV+ and 33 were BV- at enrollment. BV+ women had 2.46-fold higher aged neutrophils compared to BV- ($p=0.0114$), while antigen presenting cells (APCs) were 6-fold lower ($p<0.05$). IP-10 levels were 0.193-fold lower in nLD microbiomes than LD, while the IFNα2, IL-1α, IL-1β, and TNF-α levels were increased (1.17-fold, 4.39-fold, 9.77-fold, 2.77-fold, $p<0.05$). Six BV-related bacterial genera negatively associated with IP-10, though not passing correction individually. TNF-α positively correlated with CD14- neutrophils ($r=0.5354$, FDR adj. $p=0.0404$). Nine of 19 BV+ women responded to treatment, with three experiencing a recurrence by month 6. These findings suggest that APCs and neutrophil subtypes may contribute to improved BV treatment strategies.

Abstract 28

C9orf72 in myeloid cells prevents an inflammatory response to microbial glycogen

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Dysbiosis of the gut and neural inflammation occurs in neurodegenerative diseases such as Amyotrophic lateral sclerosis (ALS) without overt loss of gut barrier integrity or inflammation of the bowel. A hurdle in translating this knowledge into new therapies is our incomplete understanding of how bacterial species or complex microbial communities interact with host genotype to impart risk of systemic and neural inflammation. We hypothesized that commensal bacteria, which are normally well tolerated by the immune system, become neurotoxic when sensed by macrophages that experience reduction of the protein encoded at *Chromosome 9 Open Reading Frame 72 (C9ORF72),* mutation of which is the most common genetic cause of ALS. By exposing cultured macrophages and germ-free mice to bacteria that were enriched in environments where *C9orf72* loss of function mice were at risk of premature death, we identified bacterial species that were sufficient to activate a systemic and neural inflammatory response. In addition, meta-transcriptomics of fecal matter revealed an enrichment of the bacterial glycogen biosynthesis pathway in pro-inflammatory environments. Furthermore, enzymatic digestion of microbial glycogen improved the survival of *C9orf72* deficient mice and reduced macrophage cytokine production induced by pro-inflammatory bacteria and fecal matter from ALS patients with rapid disease course. Our studies provide insight into the physiologic signals that promote immunologic tolerance to commensal microbes, establish a pre-clinical platform to evaluate the neural inflammatory potential of each patient's gut microbiome and identify therapeutic strategies that may have potential to benefit ALS patients including those without a familial history of disease.

Abstract 29

Complement mediates strain-specific sensing and phagocytosis of *Debaryomyces hansenii*

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Debaryomyces hansenii is a common environmental yeast that is used in the production of agricultural products, especially cheese. It is widely believed to be safe for food use; however, our lab has shown that *D. hansenii* can be isolated from the ulcers of Crohn's disease (CD) patients, and that this microbe can delay intestinal wound healing in murine models of IBD. Ongoing work from the lab shows that different isolates of *D. hansenii* segregate into two phylogenetically and morphologically distinct clades. Clade X contains patient isolates and some food isolates, while the remaining food isolates cluster together in Clade Y.

Our lab's previous work showed that *D. hansenii* localizes to F4/80$^+$ macrophages in the intestine, raising the question of how the immune system responds to *D. hansenii*. We first measured macrophage phagocytosis, hypothesizing that sensing of *D. hansenii* would differ between the two clades, and found that Dectin-1 and CR3 mediates phagocytosis of Clade X, but not Clade Y. We next investigated how complement, a common defense against microbial pathogens, influences sensing and phagocytosis. *D. hansenii* was opsonized with normal mouse serum and C3 deposition was quantified. Despite being opsonized to a similar degree, phagocytosis of Clade X, but not Clade Y, was increased by opsonization. Interestingly, we observed opsonization of Clade X was able to rescue phagocytosis during Dectin-1 blockade. Future studies will continue to elucidate mechanisms of phagocytosis of Clade X vs. Clade Y to determine how the subsequent immune response results in delayed intestinal wound healing.

Abstract 30

Activation of the inflammatory response through IFN-γ treatment requires Krüppel-like factor 6 signaling

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Interferon-gamma (IFN-γ) is a cytokine that polarizes macrophages towards pro-inflammatory M1-like phenotypes, an important step in removing harmful pathogens and other dangerous agents. As such, understanding how the IFN-γ signaling pathway is regulated can provide insight on how to develop more targeted therapies for inflammatory diseases, autoimmune conditions, and infections. Krüppel-like factor 6 (KLF6) is a transcription factor that has also been identified as a mediator of inflammation. Previous studies have shown myeloid cell-specific deletions of *Klf6* in mouse models attenuated the development of inflammatory edema and diet-induced adipose inflammation, showing its potential for further investigation. In this study, we identified KLF6 as a key downstream signaling molecule of IFN-γ. In bone marrow-derived macrophages (BMDMs), treatment with IFN-γ induced expression of KLF6. However, when KLF6-knockout BMDMs (K6KOs) were treated with IFN-γ, they showed lower levels of downstream inflammatory signaling proteins compared to wild type (WT) cells. Gene set enrichment analysis (GSEA) of genes upregulated in response to IFN-γ in WT cells, but not in K6KOs, revealed that the genes of interest were involved in hallmark pathways of inflammation and immune cell activation. These results suggest that KLF6 expression is a necessary component of macrophage response to IFN-γ.

Abstract 31

Breastmilk Anti-Rotavirus Antibodies and Their Impact on Rotavirus Infection

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Rotavirus (RV) is a leading cause of dehydrated gastroenteritis in young children worldwide, contributing to nearly 200,000 deaths annually, particularly in low-to-middle income countries. Maternal breastmilk antibodies are a critical component to an infant's early immune defense. However, it remains unclear whether breastmilk confers genotype-specific protection or broader cross-protective immunity against multiple strains. To investigate the impact of breastmilk antibodies against wild-type RV genotypes, antibody levels against P[4], P[6], and P[8] RV genotypes will be measured using baseline breastmilk samples from 444 mothers in the Sapovirus-associated Gastro-Enteritis study in Nicaragua. We are developing a VP8 antigen-binding assay using recombinant VP8 proteins from these genotypes to quantify genotype-specific antibody responses. The assay was optimized on a Meso Scale Discovery platform to maximize sensitivity using two recombinant VP8-specific IgG monoclonal antibodies (mAbs). Binding curves for the P[4] and P[8] antigens were generated, but no binding was detected for P[6]. To determine whether this lack of binding is due to improper recombinant protein folding or an issue with the mAbs, we are propagating a P[6] genotype RV and testing an additional VP8-specific mAb clone. Once optimized, we will quantify P[4], P[6], and P[8]-specific IgA and IgG concentrations in breastmilk and assess whether these correlate with protection in breastfed infants. Future studies will involve isolating B cells from milk to examine IgA mAb specificity and breadth against various RV strains. This project will provide insight to the genotype-specific protective potential of breastmilk antibodies and how maternal immunity influences infant susceptibility to diverse RV strains.

Abstract 32

TNFRSF13B Variants Modify Adaptive Immunity to Pathogens

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TNFRSF13B or the transmembrane activator and CAML interactor (TACI) is a cytokine receptor expressed on B cells and Th follicular cells. *TNFRSF13B* governs plasma cell differentiation and contributes to defense against encapsulated bacteria. *TNFRSF13B* mutations are associated with common variable immunodeficiency (CVID) and IgA deficiency however, 98% of those carrying disruptive alleles are healthy and analysis of mutations suggests positive selection at a cost. To explore mechanisms of selection, we examined how a common *TNFRSF13B* variant (murine A144E, homologous to human A181E) determined pathogenesis by *C. rodentium*, a model of human enterohemorrhagic *E. coli*. Tnfrsf13b A144E mice resisted infection with lab strain *C. rodentium* at doses that cause disease in wild type (WT) mice. Mutant's resistance to lab strain *C. rodentium* infection is due to deficiency of secreted IgA (sIgA), as WT sIgA induced virulence *in vitro* and *in vivo*. However, when challenged with host-adapted, hypervirulent *C. rodentium*, mutant mice cleared the infection as effectively as WT mice. Mutant mice exposed to *C. rodentium* exhibited heightened immunity compared to WT mice despite decreased anti-intimin antibodies, a key virulence factor and target of protective immunity. Mutant mice produced IgG with decreased sialylation and galactosylation resulting in increased activation of complement compared to IgG from WT mice. Thus, Tnfrsf13b A144E mice exhibit phenotypic traits that induced resistance and improved adaptive immunity to enteric pathogen infection. These findings suggest *TNFRSF13B* polymorphisms are maintained by enhancing IgG effector functions inducing resistance to enteric disease at the cost of increased risk of immunodeficiency and autoimmunity.

Abstract 33

IL7 increases stem-like CD8+T cells in the tumor microenvironment

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CD8+ T cells play a crucial role in recognizing and eliminating tumor cells expressing tumor-associated antigens. Their increased number and activity can help improve the survival rate of cancer patients. However, continuous antigenic stimulation and upregulation of immune checkpoints signals including PD-1, lead to T cell exhaustion and death, allowing for tumor progression and invasion. This exhaustion limits the efficacy of immunotherapy, highlighting the importance to enhance the T cell activity within the tumors. A small subset of CD8+ T cells termed the stem- like CD8+ T cells, have the capacity to differentiate. While they express inhibitory markers like PD-1, they can differentiate into effector cells. Hence, they can help improve clinical outcomes of cancer patients. IL-7, a key cytokine in adaptive immunity, can support maintenance and proliferation of immune cells within the tumor and also drive immune cell migration into the tumor microenvironment. NT-I7, a long-acting human IL-7 molecule, was developed to enhance these effects. To investigate its effect, we injected mice with Lewis lung carcinoma cells [LLC1-GFP cells] subcutaneously. On day 7, one group of mice was injected with NT-I7 [200 ug/100 ul] and the other group received the diluent. On day 15, tumors were harvested and processed for flow cytometry. Our results show that following treatment with NT-I7, there is enhanced infiltration of $CD8^+$ T cells into the tumor while promoting a shift toward a stem-like $TCF1^+$ phenotype. There was a reduced percentage of Granzyme B^+ effector cells in the tumor tissue. These findings suggest that NT-I7 can reshape the TME and improve the efficacy of immunotherapy by increasing/expanding pool of stem-like CD8 T cells.

Abstract 34

A specific clade of *Debaryomyces hansenii* is gut-adapted and induces distinct host immune responses

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Although *Debaryomyces hansenii* is a food yeast that is generally recognized as safe for consumption, our lab identified viable *D. hansenii* within the ulcers of a subset of Crohn Disease (CD) patients. These CD isolates hindered intestinal repair in mice in a Type I interferon and CCL5-dependent manner. An important unanswered question is whether all food strains can cause disease? To test this, we isolated *D. hansenii* strains from a variety of foods. Phylogenetic analyses revealed that food strains of *D. hansenii* segregated into two clades: Clade X isolates clustered with human CD isolates, and Clade Y isolates clustered with the reference strain. We hypothesized clade variation would modulate both microbial colonization of the host and host immune responses. We observed that alike patient isolates, Clade X food isolates were propagated in vitro at 37°C and colonized the mouse gut following antibiotic pretreatment. Clade Y isolates were not propagated at 37°C nor able to colonize mice. These data support our hypothesis that the food supply carries host-adapted strains of *D. hansenii*. We next focused on how macrophages, a key cell type in *D. hansenii* exacerbated colitis, induce inflammation to diverse isolates. We observed differences in cell wall between the clades which dictated macrophage sensing through Dectin1. Ongoing efforts involve a forward genetic screen to identify the microbial genes that govern host response to diverse isolates. These studies are important to improve the safety of our food supply for patients with chronic inflammatory states in the intestine like Crohn Disease.

Abstract 35

TNFRSF13B polymorphism and maternal-fetal host defense

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TNFRSF13B encodes TACI (Transmembrane activator and CAML interactor), a receptor expressed on B-cells, T-follicular helper cells, and T regulatory cells. TACI governs B cell development in response to infection including the differentiation of plasma cells. *TNFRSF13B* is a highly polymorphic gene with 16% of individuals carrying at least one missense mutation. Some TNFRSF13B variants are associated with hypogammaglobulinemia, IgA deficiency, and increased susceptibility to certain infections. However, 98% of people with *TNFRSF13B* mutations are healthy. To investigate this paradox, we used the TACI A144E (homologous to a common human variant, A181E) mutant mouse model to study B-cell mediated immunity to an enteric pathogen, *Citrobacter rodentium.* Our work revealed that TACI A144E mutant mice resist *C. rodentium* infection due to decreased mucosal secretory IgA (sIgA) which prevents *C. rodentium* from exploiting host sIgA to induce its virulence genes. Our results thus suggest that *TNFRSF13B* polymorphisms are maintained in the population by balancing selection. Given that maternal-fetal protection against infection is one of the strongest selection pressures for gene variant retention we will investigate the impact of TACI variants expressed by the mother and/or the pups to determine protection of the newborn against common neonatal enteric infections (murine rotavirus) and according to the mother's immunological memory status.

Abstract 36

Vascular Tissue Expression and Immune Recognition of Citrullinated Self-Proteins in People with HIV

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People with HIV on antiretroviral therapy (ART) have increased levels of inflammation and have a two-fold increased risk for cardiovascular disease (CVD) compared to people without HIV (PWoH) even after adjusting for traditional risk factors. Citrullination is an inflammation-induced post-translational modification by which peptidyl-arginine is converted to peptidyl-citrulline by the removal of an imine group, creating modified self-proteins targetable by the adaptive immune system. We hypothesize that citrullination is a key process that contributes to increased CVD risk in PWH: anti-citrullinated protein antibodies (ACPAs) have been linked to increased CVD risk among the general population, and citrullinated protein-reactive T cells have been found to exhibit a phenotype similar to vascular-homing T cells that our lab has shown are more prevalent in PWH and which have also been implicated in CVD. Here, we found that PWH on ART were more likely than PWoH to have ACPAs as measured by ELISA, and more likely to have increased citrullinated-protein reactive CD8+ T cells compared to PWoH as measured by flow cytometry. We next used immunohistochemistry to measure if vascular tissues from PWH and PWoH contained possible antigenic targets for citrullinated protein-reactive CD8+ T cells. Interior carotid artery tissues from PWH had more citrullinated proteins than did carotid atherosclerotic plaques from PWoH. Additionally, we observed a trend toward differential citrullinated-protein distribution among arterial tissues of PWH. Taken together, our findings indicate that immune reactivity to citrullinated self-proteins within specific arterial tissues may be a possible mechanism contributing to increased CVD risk among PWH.

Abstract 37

NF-κB-Dependent Transcriptional Regulation of Piezo1 Mediates Bacterial Clearance of *P. aeruginosa* from the Lung

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P. aeruginosa pneumonia causes lung injury and stiffening through its virulence factors (e.g., flagellin) during pneumonia, leading to respiratory failure and death. The macrophage mechanosensitive Ca^{2+} channel Piezo1 integrates bacterial and stiffness signals to augment bacterial clearance. However, transcriptional regulation of Piezo1 during pneumonia is poorly understood. Bone marrow-derived macrophages (BMDMs) from C57BL/6J (WT) mice were treated ± *P. aeruginosa* flagellin. mRNA was measured using qPCR and chromatin binding was measured using chromatin immunoprecipitation. Flagellin increased Piezo1 mRNA levels by > 3-fold in association with increased binding of NF-κB subunit, p65 to the Piezo1 promoter and enhancer regions. Next, Piezo1 Ca^{2+} channel activity in response to agonist, Yoda1, was measured on pathophysiologic-range lung stiffnesses (1-25 kPa) using the FLIPR 5 Ca^{2+} assay. Piezo1 Ca^{2+} channel activity increased by 10-fold (Yoda1 EC_{50} from 10 μM to 1 μM) upon flagellin stimulation and required matrix stiffness which mimics infected lung (25 kPa). Phagolysosome maturation, a Ca^{2+}-dependent step in bacterial clearance, was measured using pHrodo bioparticles. Piezo1 increased flagellin-induced phagolysosome maturation > 2-fold in mouse BMDMs and human alveolar macrophages, demonstrating the importance of Piezo1 for bacterial clearance. The role of Piezo1 was evaluated *in vivo* after pneumonia was induced by intratracheal administration of a clinical strain, *P. aeruginosa* (PAM 57-15) in WT mice ± the Piezo1 inhibitor, GsMTx4. Piezo1 reduced inflammatory cell infiltration and enhanced bacterial clearance in the lungs during *P. aeruginosa* pneumonia. In conclusion, macrophage Piezo1 is transcriptionally regulated by NF-κB/p65 during pneumonia for bacterial clearance on stiffened lungs.

Abstract 38

Human cytomegalovirus G protein-coupled receptor (GPCR) UL78 regulates virus reactivation from latency

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Human cytomegalovirus (CMV) infection has a high seroprevalence (55-80%) in the US and is a major public health threat in immunodeficient individuals. After primary infection, the virus maintains latency in hematopoietic cells, followed by periodic reactivation. UL78 is one of four CMV-encoded G protein-coupled receptors (vGPCRs) and is expressed during latency; however, its function(s) remains unknown. To define UL78's contribution during latency and/or reactivation, we generated viral mutants, including a UL78 ORF deletion (UL78Δ). While all viruses maintained viral latency in hematopoietic cells, UL78Δ failed to reactivate as effectively as the wild type, suggesting UL78 is important for efficient CMV reactivation. UL78 is not known to signal via G protein, and corroborating this, we show that mutating the G protein coupling domain (UL78DRL^{AAA}) does not impact reactivation. Prior work revealed ectopic co-expression of UL78 with another vGPCR, US28, resulted in their physical interaction, which altered US28-mediated signaling. US28-mediated signaling is required for viral latency, although how US28 switches from pro-latent to pro-lytic as virus reactivates is unknown. We hypothesize UL78 is required to "switch" US28's signaling during viral reactivation. We confirmed the UL78:US28 interaction and colocalization in the context of infection. Furthermore, upon co-expression of UL78 and US28 in transfected cells, we observed an upregulation of US28-regulated (MAPK) signaling, which is silenced during latency but activated for reactivation. Taken together, our results show UL78 is required for efficient CMV reactivation, which may function to switch US28 signaling during reactivation. This work reveals novel pathways for therapeutic intervention and downstream disease.

Abstract 39

Induction of necroptosis releases MLKL's executioner domain before its RIPK3-mediated phosphorylation

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Necroptosis is required to maintain tissue homeostasis. While this form of cell death clears pathogens and cancer cells, when dysregulated, it can also cause devastating tissue damage especially in ischemia-reperfusion and chemical-induced injuries. A detailed biochemical mechanism of necroptotic execution is necessary to identify the best targets for pharmaceutical intervention to prevent necroptotic tissue damage.

Biochemically, necroptosis is tightly regulated by multiple phosphorylation events, leading to the final phosphorylation of the executioner protein MLKL by RIPK3. The exact purpose of this phosphorylation event is not well-defined but assumed to promote MLKL oligomerization and translocation to internal membranes, leading to cellular demise. However, this phosphorylation event alone is not sufficient to cause cell death. This is best demonstrated by the fact that phosphomimetic MLKL cannot cause cell death, as well as the observation that certain MLKL mutants are phosphorylated in the absence of cell death. These results suggest that, in addition to RIPK3-mediated phosphorylation, there are additional requirements for MLKL activation and subsequent necroptotic cell death.

To elucidate these additional requirements, we performed structure-function analysis and surprisingly observed that removal or displacement of the lipid binding domain increased MLKL phosphorylation. In contrast, locking the autoinhibitory state with a pharmacologic inhibitor or with an antibody mimetic prevented phosphorylation of MLKL by RIPK3. Our results are consistent with a model where the N-terminal executioner domain of MLKL is released from the brace prior to phosphorylation by RIPK3, suggesting a novel mechanism by which kinase substrates can allosterically regulate their cognate kinase.

Abstract 40

Testing of Bat STING Orthologs Reveals Species Specific Differences in STING Functionality

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Bats are thought to be reservoirs for many deadly human viruses due to having evolved a balanced immune response that controls infection while minimizing systemic inflammation. Minimization of inflammation likely evolved to reduce pathogenesis from cellular damage caused by metabolically intensive independently powered flight. The cGAS-STING pathway is important for pathogen detection by sensing cytosolic DNA from infection or cellular stress and triggers multiple immune pathways. A bat STING (bSTING) ortholog was found to have a dampened IFNb response due to a phosphosite mutation and its reversion did not relieve reduced signaling in two bat species. We hypothesize that diverse bSTING orthologs will have a range of molecular mechanisms that modulate STING activity to be less inflammatory compared to human STING (hSTING). Three bat orthologs, *E. fuscus*, *P. alecto*, and *R. aegyptiacus*, activated the IRF3-dependent reporter slightly less than WT hSTING and slightly more than phosphomutant hSTING despite having the phosphosite mutation. The bSTINGs activated autophagy at a lower rate compared to human STING and had a differential response depending on the agonist used. Induction of cell death only happened with *P. alecto,* which was less robust than hSTING. Our findings suggest bSTING orthologs have differential magnitudes of pathway activation that vary depending on agonist. To understand how bSTING orthologs functionality evolved between species we plan to test 23 orthologs. As our ability to scale protein variant screening increases, we will gain a deeper understanding of how bats evolved immune adaptations to allow for tolerance of infections.

Abstract 41

Myosin 18A regulates central and peripheral B cell tolerance

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Autoimmune diseases impact roughly 50 million Americans and cause a plethora of chronic, debilitating symptoms that are physically, mentally, and financially burdensome. Autoantibodies stem from autoreactive B cells that escape the body's mechanisms of tolerance. Actomyosin interactions are associated with B cell activation, yet mechanisms connecting B cell cytoskeletal dynamics to tolerance remain poorly characterized. We previously generated a B cell-specific deletion of Myosin18A (Myo18A BKO) and observed a significant increase of serum autoantibody concentrations, indicating that B cell tolerance may be affected by the loss of Myo18A. We performed single-cell RNA sequencing on bone marrow cells and discovered a higher frequency of immature B cells in Myo18A BKO mice. Hallmark and Reactome analyses identified significant de-enrichment of the apoptosis and DNA repair pathways, noting that key mechanisms of central tolerance – clonal deletion and B cell receptor (BCR) light chain editing – may be defective in Myo18A BKO mice. Further, mature Myo18A BKO B cells displayed greater AKT phosphorylation upon BAFF stimulation, a crucial cytokine for peripheral B cell survival. Mature Myo18A BKO B cells showed greater differentiation into antibody-secreting cells upon TLR7 and BCR stimulation. Bulk RNA sequencing of mature, naïve B cells indicated a significant increase in expression of genes associated with ER stress and the unfolded protein response, indicating that ablation of Myo18A causes B cells to be poised for plasma cell differentiation even in the absence of external stimuli. Our results indicate that Myo18A regulates multiple pathways that are essential for B cell selection, survival, and differentiation.

Abstract 42

Determining the genes and pathways in M2-like macrophages responsible for subverting CD4+ T-cell activation upon infection with Mycobacterium tuberculosis

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The bacteria Mycobacterium tuberculosis (Mtb) specializes in evading the immune response and requires efficient CD4+ T-cell activity protection. Recent evidence in mice shows that recognition of infected macrophages by Mtb-specific CD4+ T-cells is imperative for control. We recently published that M1 but not M2-like macrophages efficiently activate Mtb-specific memory CD4+ T-cells upon infection with Mtb. However, the mechanisms remain unknown. We performed RNA sequencing on human M1 and M2-like macrophages exposed to either virulent Mtb infection, γ-irradiated Mtb, or Mtb whole-cell lysate. Since M2-like macrophages treated with either Mtb lysate or irradiated Mtb were able to activate CD4+ T-cells, these conditions served as important controls. 765 differentially expressed genes (DEGs) were unique to M2like Mtb-infected macrophages compared to M1 macrophages. We also performed RNA sequencing on Mtb infected and non-infected BAL macrophages. Mtb-infected BAL macrophages shared 102 DEGs with infected M2-like macrophages. Pathway enrichment and gene ontology of these genes identified IL-10 Receptor Signaling, Type I Interferon signaling and the NADPH Oxidase Complex as key components of the M2 and lung macrophage response. We curated a list of these key genes that represent candidate inhibitory pathways of T-cell activation. Current work is focused on knockdown of validated DEGs in M2-like macrophages using RNAi to examine CD4+ T-cell activation responses upon Mtb infection. Our results will identify key genes responsible for the subversion of CD4+ T-cell activation by Mtb-infected M2-like macrophages and will also expose key interactions facilitating CD4+ T-cell recognition of infected macrophages, the niche cells for Mtb infection.

Abstract 43

TGFBRAP1 Overexpression Inhibits SARS-CoV-2 and Filovirus Entry by Modulating Endosomal Trafficking

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The CORVET and HOPS complexes are hexameric proteins involved in endosomal trafficking. They contain four common core proteins and two complex specific subunits. VPS8 and TGFBRAP1 (VPS3) are CORVET specific subunits that activate early to late endosomal fusion through associations with Rab5. Conversely, HOPS specific subunits induce late endosomal-lysosomal fusion through Rab7 interactions. Preliminary data from a gene activating transposon mutagenesis screen for Ebola virus entry restriction suggested that TGFBRAP1 is antiviral. It is known that the HOPS complex is required for filovirus entry because they must be trafficked to late-endolysosomal compartments to induce fusion. SARS-CoV-2 undergoes similar trafficking when cells lack TMPRSS2, a surface protease. When TGFBRAP1 and VPS8 are overexpressed in HEK293T cells, HOPS complex formation is inhibited. The impact of the proportions of CORVET and HOPS complexes has not been studied in the context of virus entry. HEK293T cells overexpressing TGFBRAP1 and ACE2 were infected with single cycle lentiviral particles pseudotyped with glycoproteins from SARS-CoV-2, Ebola, Marburg, LFV*, LCMV*, and *VSV* (**low pH dependent viruses)*. We confirmed that TGFBRAP1 overexpression inhibits SARS-CoV-2, Ebola, and Marburg entry, but LFV, LCMV, and VSV entry was unaffected. This phenotype is dependent on the presence of TGFBRAP1 because siRNA knockdowns increased Ebola and Marburg infectivity. We are currently trying to understand the impacts of TGFBRAP1 expression and other CORVET/HOPS specific proteins in endosomal trafficking by studying their impact on viruses that enter through this compartment. This will allow us to preliminarily determine the role of CORVET-HOPS conversion during infections.

Abstract 44

Nitric oxide plays a crucial role in regulation of Cytochrome P450 activities by controlling the allocation of heme within the cell

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The heme-containing enzymes cytochrome P450 (CYP) play crucial roles in various facets of human biology and vital life functions including the immune response and liver function, but the mechanisms of cellular heme delivery and insertion that facilitate their maturation into functional forms remain largely unclear. Nitric oxide (NO) generated during immune activation in animals was previously found to have a significant impact on the catalytic activities of CYP's. Since their activities rely on their heme contents, we investigated the potential effects of NO on CYP heme allocation levels in mammalian cells. We utilized several cell lines that were transiently transfected with two different CYP enzymes (CYP3A4 or CYP2D6) to assess their activities, heme contents, and expression levels in relation to cell NO exposures. Our research reveals that NO operates with a bimodal effect. Within a specific low exposure range, NO facilitates the allocation of cellular heme into the heme-deficient CYP3A4/2D6 populations, resulting in a substantial (3-fold) increase in their heme contents and activities. However, when NO exposure surpasses this lower range, it loses its positive impact and ultimately lowers CYP heme levels and activities. This bimodal effect was evident when NO was either released from a chemical donor or was produced endogenously by immune-stimulated macrophages. The expression levels of CYP proteins remained unaffected by NO. In addition, we also found that the NO-driven heme allocation into CYP3A4/2D6 involves heme delivery from a GAPDH-heme complex in the cell and the functional activity of cell chaperone Hsp90. Together, our findings indicate that NO has a crucial role in modulating cellular CYP3A4/2D6 activities through a bimodal control of their cell heme allocation. The effect of NO on CYP heme contents potentially explains how immune activation alters CYP activities and leads to changes in metabolism of drugs and generation of immune-active prostaglandin metabolites.

Abstract 45

Macrophage TRPV4 Drives Myofibroblast Differentiation through Activation of TGF-β

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Evidence suggests that macrophage-fibroblast interaction can drive organ fibrosis. Myofibroblast differentiation is a key process that drives fibrosis in the lung and multiple organs that requires both a mechanical signal and soluble factors. We have shown that the mechanosensitive calcium channel, TRPV4, drives myofibroblast differentiation, macrophage activation, and experimental pulmonary fibrosis. In the setting of emerging data implicating immune processes in fibrosis, we sought to determine if TRPV4 mediates pro-fibrotic macrophage-fibroblast crosstalk. To test this, c**onditioned media (CM) was collected from** WT and *Trpv4* KO **b**one marrow-derived macrophages (BMDMs) plated on pathophysiologic-range substrate stiffnesses (1kPa-normal lung, 8-25kPa fibrotic lung) or polystyrene (10^6 kPa). WT CM transferred to WT mouse lung fibroblasts (MLFs) induced myofibroblast differentiation and collagen-1 production, an effect that was lost with KO CM. This suggests a TRPV4-dependent pro-fibrotic macrophage secretory factor. Upon treatment of MLFs with the TGF-βR kinase inhibitor (SD208) and TGF-β immunodepletion in macrophage CM, myofibroblast differentiation was abrogated, indicating TGF-β is the likely pro-fibrotic factor. Surprisingly, total TGF-β by ELISA was similar in WT and *Trpv4* KO BMDM CM, however, the active fraction of TGF-β was reduced in *Trpv4* KO BMDM CM. TGF-β activation was dependent on extracellular calcium uniquely in WT BMDM CM, but not in *Trpv4* KO BMDM CM. For the first time, our study links physiologic matrix stiffness sensing, calcium influx through TRPV4, and activation of TGF-β in macrophages with resultant pro-fibrotic fibroblast to myofibroblast differentiation. This provides further impetus to develop existing TRPV4 inhibitor for therapeutic intent.

Abstract 46

Harnessing breastmilk immunity to protect neonates against influenza virus

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Newborns and infants are particularly vulnerable to viral infections due to the immaturity of their immune systems. Consequently, strategies that enhance maternal and neonatal immunity are essential for safeguarding their health. Maternal immunization is a valuable strategy to amplify virus-neutralizing antibodies in both blood and breast milk, ensuring optimal passive immunity transfer to the infant. Our group previously demonstrated that, in pregnant and lactating ferrets, intranasal immunization with an adenovirus type 5 (Ad5) H1N1 hemagglutinin (HA) vaccine administered two weeks before and two weeks after birth significantly increased influenza-specific antibody levels and neutralizing activity in both blood and milk, compared to oral and intramuscular immunization at the same intervals. One caveat of relying on maternal active immunity to confer passive protection to breastfeeding neonates is the time required for the mother to mount an effective immune response. In contrast, passive immunity can provide immediate, pathogen-specific antibody transfer. To evaluate whether passively transferred breastmilk IgA antibodies can directly protect suckling neonates from influenza infection, we generated an HA-specific, ferretized dimeric IgA monoclonal antibody (mAb). This engineered dimeric IgA chimera comprises two human Fab regions specific for the influenza HA head domain, two ferret IgA Fc regions, and a ferret J chain that links the two monomers. Following production and validation of the mAb's structure and antigen specificity, its transfer will be tracked from the circulation in lactating dam to their suckling kits. Future studies will assess the protective efficacy of these passively acquired antibodies in suckling kits challenged with H1N1 virus.

Abstract 47

Expression of soluble guanylate cyclase (sGC) and its ability to form a functional heterodimer are crucial for reviving the NO-sGC signaling in PAH

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In order to determine the underpinnings of a dysfunctional NO-sGC signal pathway which occurs in pulmonary arterial hypertension (PAH), we investigated pulmonary arterial smooth muscle cells (PASMCs) derived from PAH patients. We found low expression of sGC, a poor sGCα1β1 heterodimer and this correlated with low expression of its facilitator chaperone, hsp90. Treating PASMCs overnight (16 h) with low micromolar doses of a slow-release NO donor DETANONOate, reinstated the sGCα1β1 heterodimer and restored its NO-heme-dependent activity. Transwell co-culture of HEK cells stably expressing eNOS with PAH PASMCs also restored the sGC heterodimer and its heme-dependent activity with sGC stimulator, BAY 41-2272. To determine whether the dysfunctionality in the NO-sGC pathway stems from a dysfunctional eNOS producing negligible NO, we did transwell co-cultures of pulmonary arterial endothelial cells (PAECs) with PASMCs. Our results indicated that PAECs from both control and PAH samples when activated for eNOS restored both sGC heterodimer and its heme-dependent sGC activity in the corresponding PASMCs, suggesting that PAECs from PAH can also generate NO. In line with these results expression of eNOS, its support chaperon hsp90, its specific kinase Akt, p-Akt or post-translational modifications (PTMs) like OGlcNAc or phospho-tyrosine were unchanged in PAH relative to controls. Additionally, there was uniform expression of Hbα/β and Mb in PASMCs or PAECs in PAH or controls and these globins can effectively scavenge the eNOS generated NO, as there was evidence of strong eNOS-Hb/Mb interactions. Our studies suggest that factors such as globin NO scavenging, along with vascular remodeling in PAH can cause hampered vasodilation, which in the face of poor NO levels as occurs in PAH are additional impediments for effective vasodilation. However importantly our studies suggests that future therapies can use low doses of NO along with sGC stimulators as a potential drug for PAH subjects.

Abstract 48

Mycobacterium tuberculosis (Mtb)-infected cells are recognized by autologous memory CD4+ T cells

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Macrophages serve as the primary niche for *Mycobacterium tuberculosis* (Mtb), the etiologic agent of tuberculosis (TB). The animal models of TB have helped the field to establish that CD4+ T cells are critical for protection. However, only a fraction of T cells present in the lung of infected mice recognized Mtb-infected macrophages *ex vivo*. Infection assays using fluorescent reporter strains of Mtb reveal that not every macrophage exposed to Mtb becomes infected, herein termed as bystanders (BYSTs). Little is known about how primary human CD4+ T cells respond to BYST macrophages exposed to Mtb infection. Therefore, our study explored the overarching hypothesis that BYST macrophages acquire Mtb antigens and are recognized by CD4+ T cells in the context of Mtb infection. Using a fluorescent reporter strain of virulent Mtb for flow-sorting assays and transwell plates, we infected primary human monocyte-derived macrophages (MDMs) with Mtb and found a subset of memory CD4+ T cells that upregulated activation-induced markers in response to autologous BYST macrophages in a TCR:pMHCII-dependent manner. Given prior reports suggesting the presence of Mtb antigens in exosomes released by infected cells, we tested whether this type of extracellular vesicle facilitated the recognition of uninfected macrophages. Our results also revealed that host exosomes released during infection did not significantly impact CD4+ T cell activation in response to exosome-treated macrophages. Our findings suggest that non-infected macrophages present Mtb antigens to CD4+ T cells, but exosomes are not the primary transfer mechanism. We are currently investigating alternative antigen transfer.

Abstract 49

The effect of altered B cell metabolism on vaccine response in obesity

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Obesity is a serious and common chronic disease. With more than 2 in 5 U.S. adults having obesity, the impact of metabolic disease on immunity has reached a critical point. Strong evidence indicates individuals with metabolic diseases, such as obesity, do not have appropriate humoral immune responses. In addition, immune responses to vaccines wane faster in individuals with obesity. Humoral immunity relies on production of short- and long-lived plasma cells (PCs) as well as memory B cells and the proper balance of these depends on B cell-intrinsic metabolism. We hypothesize that increased basal metabolic activity in naïve B cells in metabolic disease biases them toward rapid PC differentiation rather than germinal center reactions, leading to loss of high affinity long-lived PCs and memory B cells. Preliminary data show wild type mice on high fat diet produce fewer long-lived, high affinity PCs upon vaccination. Furthermore, gene expression analysis show that PCs produced in animals on high fat diet had a more immature phenotype. Future studies will determine differentially activated metabolic pathways and perform functional assays to assess cell-intrinsic effects of diet-induced obesity on B cell responses to various vaccine platforms. By modulating the adjuvant strength and antigen persistence, we hypothesize that we can tune this response to optimize humoral immune responses in the context of obesity.

Abstract 50

Inverse-vaccines for Rheumatoid Arthritis Re-establish Metabolic and Immunological Homeostasis in Joint Tissues

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Rheumatoid arthritis (RA) causes inflammatory and metabolic imbalances in tissue, which then exacerbates inflammation in affected joints. Therefore, restoring tissue homeostasis is necessary for the remission of RA symptoms. In fact, the changes in immunological and metabolic tissue homeostasis at different stages of the disease is not well understood. Herein, the changes in the immunological metabolic profiles in different stages of RA namely, early, intermediate, and late stage was examined. Moreover, the efficacy of the microparticle inverse-vaccine, paKG(PFK15+bc2) to restore immunological and metabolic tissue homeostasis at different stages of the disease was also investigated. Analysis of the immune cell profiles revealed that there was a significant decrease in the activation of pro-inflammatory immune cells while a remarkable increase was seen in regulatory T-cell populations in the intermediate and late stages of RA in the inverse-vaccine treated group as compared to no treatment. Also, it was determined that glycolysis in the spleen was normalized in the late stages of CIA, which was similar to no disease tissues. Using metabolomics we identified, key metabolites UDP-glucuronic acid and L-Glutathione oxidized that were significantly altered between treatment groups, and thus might provide new druggable targets for RA. We also employed flux metabolic modeling of the metabolomics data to identify amino acid and carnitine pathways as the central pathways affected at the tissue-level in CIA as the disease progresses. Overall, this study shows that the inverse-vaccines initiate early re-establishment of homeostasis and persists through the disease span.

Abstract 51

Interleukin-27 Regulates B and T Cell Responses in Transplantation and Shapes Donor-Specific Alloantibody Production

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Introduction: Antibody-mediated rejection (AMR) remains a challenge in transplantation, necessitating new strategies to prevent donor-specific alloantibody (DSA) generation. Interleukin-27 (IL-27), a cytokine of the IL-12 family produced by dendritic cells (DCs) and macrophages, shares signaling pathways with IL-6. This study evaluates IL-27's role in modulating immune responses in transplant models and its impact on graft survival.

Methods: Fully MHC-mismatched heterotopic heart transplants (BALB/c → B6.WT or B6.IL-27R-/-) were performed, with DSA measured by ELISA on days 7, 14, and 21 posttransplant, and immune cell populations analyzed by flow cytometry. In a skin graft model, B6.WT recipients received anti-IL-27 or control IgG therapy.

Results: IL-27 was produced by DCs, macrophages, and B cell subsets, including germinal center and plasma cells. B6.IL-27R-/- recipients showed reduced B220+ populations, particularly transitional and follicular B cells, while plasma cells were significantly lower. T cell analysis revealed decreased CD3+, CD8+, CD4+ naïve, and effector T cells in IL-27R-/- recipients. Additionally, CD11b+ and CD11b+CD11c+ populations were reduced. By day 21, B6.IL-27R-/- recipients had significantly lower DSA levels. Anti-IL-27 therapy improved skin graft survival and reduced effector T cells.

Conclusions: IL-27 modulates humoral and cellular alloimmune responses, influencing B and T cell subsets. Targeting IL-27 suppresses DSA production and enhances graft survival, supporting its potential as a therapeutic target in transplantation.

Abstract 52

Investigating the role of intestinal epithelial cell (IEC)-derived gasdermin C (GSDMC) in the regulation of IL-33 and its impact on chronic intestinal inflammation

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Gasdermins are a family of structurally-related proteins known for their role in pyroptosis, or lytic cell death; however, non-lytic functions for these proteins are now well-recognized. *In vivo* GSDMC function has primarily been studied during intestinal helminth infection, wherein GSDMC is upregulated and has been implicated in the extracellular release of IEC-derived IL-33, leading to mounting of Th2 immune responses towards worm expulsion. Notably, while IL-33 is recognized as a secreted cytokine, it also localizes to the cell's nucleus, and purportedly acts as a nuclear transcription factor; the mechanism of IL-33 nuclear sequestration, however, is unclear. Therefore, the aim of this study is to determine whether GSDMC facilitates nuclear translocation and sequestration of IL-33 during chronic intestinal inflammation. Our preliminary results show that in ileitis-prone SAMP mice, GSDMC is highly expressed in IECs, with robust upregulation of *Gsdmc2-4* in SAMP vs. AKR (control) ilea, which further increases with age as disease becomes more severe. Immuno-fluorescent imaging shows co-expression of GSDMC and IL-33 in ileal IECs from highly inflamed areas, with scant staining in areas of low inflammation in SAMP, and none in non-inflamed AKR mice. GSDMC accumulates at the nuclear membrane, and IL-33 in the nucleus, of IECs. Finally, *in vitro* stimulation of the human colonic epithelial cell lines, HT-29 and Caco-2, with TNF or IL-1β vs. vehicle, leads to increased IL-33 in whole cell lysates, but very little secreted IL-33. Together our results suggest that GSDMC may play a role in nuclear IL-33 sequestration during chronic intestinal inflammation.

Abstract 53

Gut Microbiome-Dependent Th17 Skewing Drives Sex Differences in a Murine Model of Crohn's Disease-like Ileitis

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Inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease (CD), is a chronic gastrointestinal disorder with an unclear etiology. Dysregulated immune responses, along with genetic susceptibility and environmental factors such as the gut microbiome, are key contributors to IBD pathogenesis. Sex-based disparities in IBD patients have been reported, with females (F) experiencing earlier onset and heightened disease severity versus males (M). Similarly, specific pathogen-free (SPF)-raised SAMP1/YitFc (SAMP) mice, an established model of CD-like ileitis, display earlier onset and increased disease severity in Fs versus Ms, with differences attenuated in germ-free (GF) SAMP. To investigate potential host immune mechanisms by which the gut microbiome influences sexual dimorphism in SAMP, cells from draining mesenteric lymph nodes (MLNs) of SPF versus GF SAMP were immunophenotyped by FACS and activated *in vitro*, with supernatants assayed via a Luminex-based Th1/Th2/Th17/Th22/Treg panel. SPF SAMP-M exhibited increased frequency of $CD4^+Foxp3^+$ Tregs versus SAMP-Fs, a difference abolished in GF SAMP. A predominant Th17, versus Th1 and Th2, immune profile was observed in SPF SAMP-F versus -M, but not in GF SAMP. Preliminary experiments investigating the cellular source of IL-17 indicate the ratios of either Th17 cells or IL-17-producing Tregs to conventional Tregs is greater in SPF SAMP-F versus -Ms. Furthermore, no differences were detected in absolute numbers of Th17-producing $\alpha\beta^+$ T cells, while a trend toward expansion of IL-17A/F-expressing $\gamma\delta^+$ T cells was observed in SPF SAMP-F versus -M. Our findings suggest that sexual dimorphism in ileitis-prone SAMP is microbiome-dependent and may preferentially drive Th17 immune responses in SAMP-F versus -M.

Abstract 54

Differential gasdermin B (GSDMB) isoform usage and function in intestinal goblet cells

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Gasdermins (GSDMs) were originally described for their role in pyroptosis, and their dysregulation has been reported in chronic inflammatory disorders, including inflammatory bowel disease (IBD). Our group described increased epithelial-derived GSDMB mainly in colonocytes/crypt top colonocytes from ulcerative colitis (UC) patients; however, upregulated *GSDMB* is also observed in goblet cells (GCs) from inflamed UC tissues. The aim of this study is to determine GSDMB protein isoform expression and usage in the established GC lines, LS174T and HT29-MTX. Preliminary data indicate differential expression and abundance of isoform-specific *GSDMB* in HT29-MTX (mainly GSDMB-416 and -407) vs. LS174T (mainly GSDMB-403) cells, suggesting potential differences in GSDMB-dependent GC functions. In fact, genes related to mucin secretion, including *LYZ*, are differentially regulated in IFNγ-stimulated native vs. *GSDMB*$^{-/-}$ LS174T cells, while genes associated with anti-microbial (AMP) biosynthesis/secretion, such as *REG4*, are differentially regulated in HT29 cells +/- MTX. To begin characterizing the role of GSDMB and its impact on mucin secretion, we analyzed mice overexpressing GSDMB-411 specifically in IECs (GSDMB-411$^{IEC\text{-}Tg}$), with results showing that GSDMB-411$^{IEC\text{-}Tg}$ vs. control mice produce and release copious amounts of mucus, with robust accumulation and aberrant mucin release, even when unchallenged. This phenotype is recapitulated in *ex vivo* organoid cultures from GSDMB-411$^{IEC\text{-}Tg}$ mice after stimulation with IFNγ, which induces re-distribution of both GSDMB and *MUC2*. Together, our results indicate potential functional relevance of GC-expressing GSDMB and its varying isoform(s), specifically regarding mucin and AMP biosynthesis and secretion, which could provide insights into the role of GSDMB in IBD pathophysiology.

Abstract 55

Furthering our understanding of how human antibody epitopes on Apical Membrane Antigen 1 are related to clinical protection from P. vivax malaria: Preliminary data & study design

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One-third of the human population, predominately in South America and Southeast Asia, are at risk of contracting *Plasmodium vivax* (Pv) malaria. Apical Membrane Antigen 1 (AMA1) is a surface-expressed essential invasion protein that binds to Rhoptry Neck Protein 2 (RON2), an interaction utilized by both sporozoites and merozoites during host cell invasion. PvAMA1 is a highly immunogenic surface antigen, and those individuals with elevated antibody titers to AMA1 are associated with reduced risk of malaria. Developing novel Pv-specific therapeutic options such as monoclonal antibodies is vital to decreasing the worldwide burden of Pv. We have identified one potent PvAMA1-specific human monoclonal antibody (humAb), 826827, that binds to the conserved epitope to which RON2 interacts with AMA1. We hypothesize that individuals that acquire antibodies against this specific epitope on Domain 1 of AMA1 correlate with protection from Pv infection compared to those who lack effective antibodies that bind to this epitope. To answer this question, longitudinal cohorts from Pv endemic regions are being analyzed and antibodies in sera are being competed against 826827 via ELISA to determine if individuals in endemic regions are naturally creating antibodies that bind to PvAMA1 better than 826827. Preliminary data show that around 20% of individuals from the high transmission region of Papua New Guinea display significant blocking of 826827. There is a correlation between 826827-blocking and Pv endemicity that has been observed.

Abstract 56

CAR T-Cell Resistance in Multiple Myeloma Using a Novel Human Bone Marrow Chip

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Adoptive transfer of chimeric antigen receptor (CAR) T-cells has shown promising results in treating r/r-MM such as anti-BCMA CAR T-cell therapy. Unfortunately, high relapse rates in long-term follow-up patients remain a formidable challenge. Surprisingly, little is known about the role of the BM niche in CAR T-cell therapy failure in r/r-MM patients. Standard 2D models often fail to replicate the complexities of the disease microenvironment, limiting their ability to reveal relevant functional differences. Preclinical animal models permit in vivo studying of CAR T-cell function, but apparent discrepancies between preclinical and clinical results have raised major concerns about the predictive value of these models due to their lack of human stroma or immune components. Taking advantage of this technical innovation, we are working to define the molecular and cellular determinants of CAR T-cell therapy failure for r/r MM patients via real-time and chronological monitoring of CAR T-cell dynamics within an in vitro humanized organotypic MM BM niche as a therapeutic screening platform. Our unique device architecture utilizes individual compartments for fibroblasts, the endosteal region (osteoblasts), and the medullary region (ECs, HSCs, MSCs, MM) to mimic the cross section of bone marrow microenvironment. The MM BM chip cellular landscape has been validated via immunofluorescence. Through scRNA sequencing analysis, we used our model to show that monocytes and macrophages in MM display an M2 phenotype with upregulated immunosuppressive pathways such as Wnt/β-Catenin signaling compared to healthy BM chips within 14 days of culturing the device.

Abstract 57

A Novel Bioinformatic Method to Trace Donor-specific B Cell Evolution in Transplant Recipients

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Donor-specific B cell responses (DS-Bs) are a major hurdle to the success of organ transplantation. All recipients develop DS-Bs but only a few undergo antibody-mediated rejection (AMR). We found that common variants of the Transmembrane Activator and Calcium Modulator Interactor (TACI) are associated with AMR. We hypothesize TACI variants determine pathogenic DSB cell evolution eventuating in rejection.

We analyzed DS-B cell immunoglobulin HC gene sequences obtained from PBMCs, by panning on lightly fixed donor-derived fibroblasts, in 10 recipients of living kidney donors. isolated before and after transplantation at 3, 6 and 12 M. IgH sequence high-quality reads were generated using pRESTO from raw FASTQ files. Assignment of V(D)J genes was done by Change-O using IgBLAST annotations. Only identical sequence-aligned IgHs observed in more than one instance were retained for downstream analysis. A neighbor-joining tree rooted on the germline was generated to represent clonal lineage relationships. For each clone, the Hamming distance between the consensus sequence and each mutated sequence within a clone was computed. Comparison between hamming distance distribution post and pre-transplantation did not change significantly or became narrower after transplantation in 3 of the 10 samples, including 2 recipients with P251L TACI. In contrast, the distribution of the hamming distances widened after transplantation in the 7 recipients that maintained healthy graft function throughout the post-transplantation period. Our findings suggest that DS-B cell intra-clonal evolution is associated with graft health and that TACI variants determine DS-B cell intra-clonal evolution and in this way the likelihood of AMR.

Abstract 58

Intestinal stem cells enhance mucosal immunity through apoptotic body phagocytosis

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Modulation of immune tone at mucosal surfaces is essential for maintaining homeostasis while responding to emerging threats. While efferocytosis typically resolves inflammation, our work reveals a paradigm in which intestinal stem and progenitor cells (ISCs) actively phagocytose apoptotic bodies to enhance local immune responses. The idea to explore this concept arose from findings published in the 1970s when pioneers of the field observed that ISCs engulfed apoptotic bodies and traced the remnants into all major adult lineages of the intestinal epithelium. Using 3D spheroid cultures enriched for ISCs and apoptotic bodies derived from newly developed immortalized mouse jejunal and colonic epithelial lines, we found that ISCs along the intestinal tract engulfed apoptotic bodies. Live spheroid imaging and robust quantification using a deep learning-based behavior segmentation tool showed that ISC uptake depends on concerted recruitment of apoptotic bodies and directed spheroid migration. Pharmacologic inhibition of diverse pathways and enhanced uptake via C3 opsonization suggests a phagocytic mechanism. Confocal and transmission electron microscopy revealed that uptake involves actin-driven basolateral membrane protrusions, with apoptotic bodies trafficked into large $LAMP1^+$ vesicles for degradation. Notably, ISC uptake induced TNF production that enhances $CD4^+$ T cell activation. *In vivo*, mice treated with the genotoxic thymidine analog EdU to selectively ablate crypt cells showed $LAMP1^+$ vesicle formation in surviving ISCs and enhanced immune activity in the absence of barrier breach. These findings support a model in which ISC-mediated phagocytosis of apoptotic bodies acts as an apex surveillance mechanism that exists in every intestinal crypt to bolster mucosal immune readiness.

Abstract 59

STAT1 gain-of-function (GOF) mutations disrupt the conformational balance of STAT1 and lead to immunodeficiencies

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Signal transducer and activator of transcription 1 (STAT1) is crucial for immune responses. STAT1 deficiency primarily causes vulnerability to bacterial and viral pathogens. Paradoxically, individuals with STAT1 gain-of-function (GOF) mutations exhibit both immunodeficiency and autoimmunity. To date, over 140 variants have been identified worldwide that generally enhance STAT1-dependent immunity but with varying clinical phenotypes. This variability, along with the lack of targeted treatments, highlights the urgent need to elucidate the mechanisms by which STAT1 GOF mutations drive immune dysregulation.

Cytokine-induced tyrosine phosphorylation of STAT1 drives a conformational shift of the STAT1 homodimer from an "inactive" to an "active" form. The latter promotes STAT1 nuclear translocation and transcription of target genes. Previous work suggested the GOF mutations could disrupt the "closed" state, promoting STAT1 hyperactivation. However, conventional approaches are limited in measuring the detailed, dynamic conformational changes. Here, we introduced a real-time, live-cell technique called NanoBiT to address this challenge.

Based on the real structure of STAT1 homodimer, we designed this system to detect both states of STAT1 homodimers. Our results revealed tested GOF mutations indeed disrupt the "inactive" state to varying degrees. Furthermore, our system successfully monitored STAT1 conformational dynamics, illustrating distinct kinetic patterns of these changes across variants in a high-throughput manner. These kinetic patterns positively correlate with clinically observed STAT1 activity levels. We plan to expand our research to test all variants aiming to establish correlations between conformational preferences, transcriptional activity, and clinical outcomes. This work will ultimately help provide patients with more accurate prognoses and personalized treatments.