Case Western Reserve University School of Medicine

Department of Pathology

19th Annual Immunology Retreat

Friday, May 1, 2026
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 19th 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 welcome Dr. Craig Maynard as our Keynote Speaker. Dr. Maynard is a world leader and expert on inflammatory bowel disease and the impact of the microbiota and their varied interactions with both host antibodies and the mucus layer. We are excited to hear more about his work and to welcome him to our community and the beautiful Botanical Gardens!

Finally, I would like to personally thank everyone on the planning committee: Drs. Wendy Goodman, Anna Bruchez, Michael Freeman, Brian Gaudette, and Stephanie Langel. I'd also like to thank the administrative staff, especially Gail Stringer, Andrea Shellenberger and Melanie Prestage, 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

2026 Immunology Retreat Keynote Speaker

Craig L. Maynard, PhD

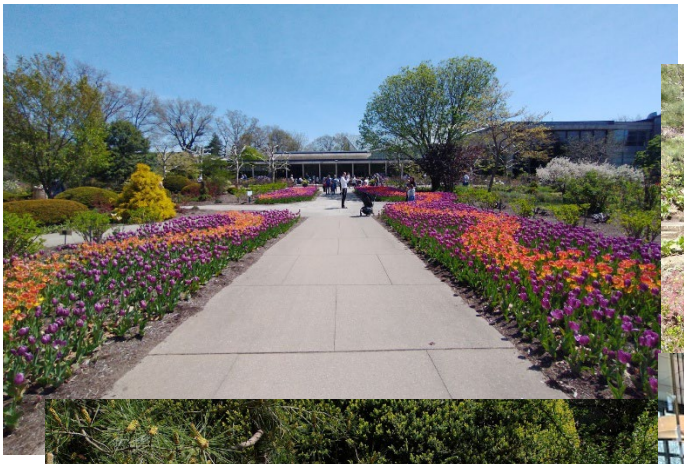
Associate Professor
Department of Pathology, Division of Molecular and Cellular Pathology
Department of Medicine, Division of Gastroenterology and Hepatology
Heersink School of Medicine
University of Alabama at Birmingham
Birmingham, Alabama, USA

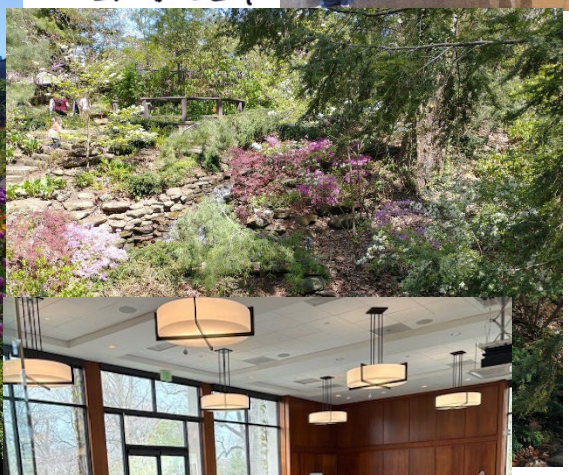
Dr. Maynard earned his bachelor's degree from Midwestern State University in 2001 and his PhD at the University of Alabama at Birmingham (UAB) in 2007. His initial Assistant Professor position began in 2015 at UAB Heersink School of Medicine and was later promoted to Associate Professor in 2022. In addition to his primary research, he serves as the Scientific Director for the Gnotobiotic (germ-free mouse) Core Facility and the Co-Director of the Immunology Theme within the Graduate Biomedical Sciences PhD Program.

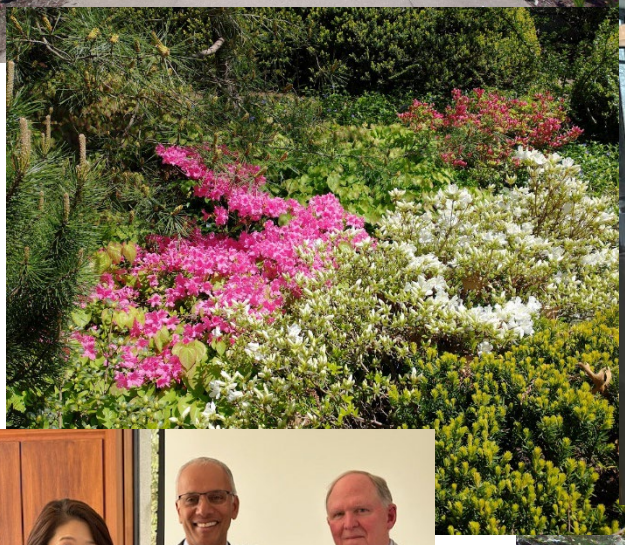
Dr. Maynard's research studies focus on inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), and the role played by the microbiota. The Maynard lab has three major directions. First, they work to understand the mechanisms regulating the activity and functions of gut mucus-associated microbes. More specifically, they are currently exploring whether and how microbial genomic diversity impacts the functions of specific mucus-associated organisms in health and under inflammatory conditions. Second, they are interested in the impact of anti-commensal antibodies in host-microbiota mutualism. They are exploring the importance of T-dependent antibodies for the regulation of gut immune homeostasis, and the synergy that exists between these antibodies and other immune pathways to continually promote immune homeostasis and prevent deleterious responses to benign commensal organisms. Finally, they investigate mucus-dependent regulation of susceptibility to inflammation and colorectal cancer. Ongoing work in the lab is exploring how components of the colon mucus layer interface with the mucus-associated microbiota in health and disease, impact barrier restitution following an inflammatory insult, and prevent chronic activation of the epithelium to limit epithelial hyperproliferation.

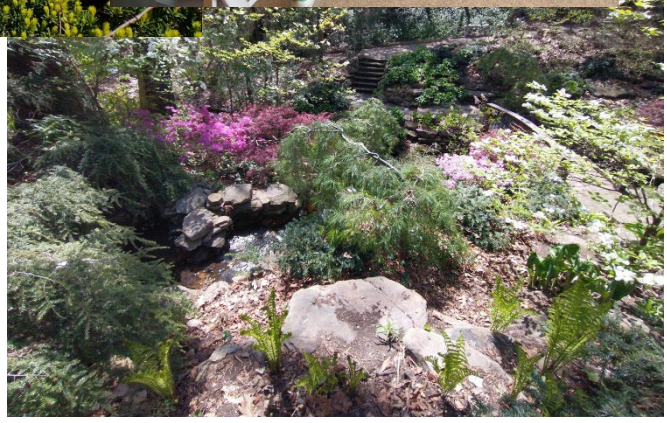
Our Retreat at the Cleveland Botanical Gardens

By my count, this retreat will be the sixth one held at the beautiful Cleveland Botanical Gardens. The venue is a wonderful way to enjoy nature while talking science in a relaxed and warm setting that is separate enough from our day-to-day to feel like a real *retreat*. I have taken some pictures over the years, but I want to invite attendees to take their own pictures of the venue and the event (perhaps some with participants and some of the gardens) and send the best ones to me (brian.cobb@case.edu) for possible inclusion in future retreat program booklets. Here are some from my collection to get this started!

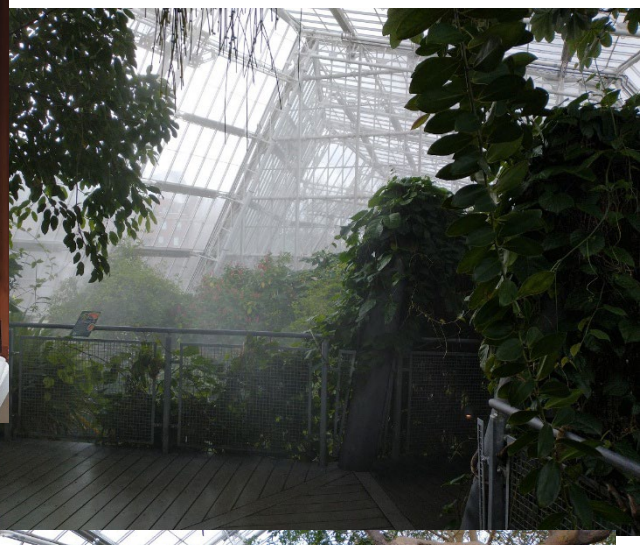


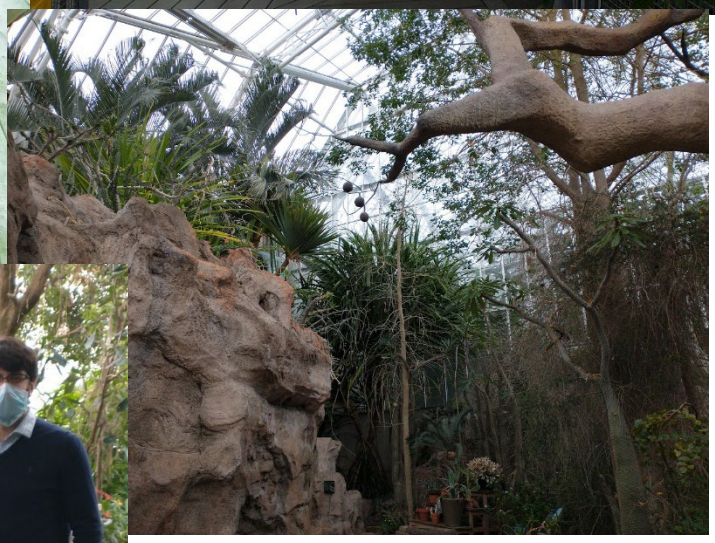


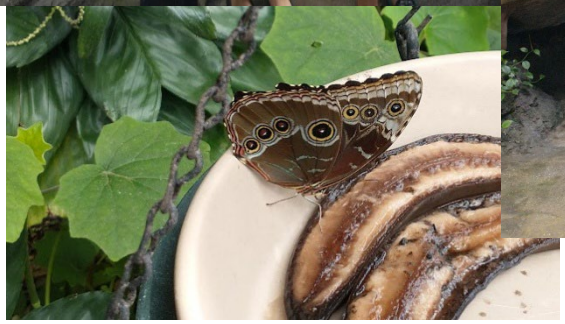





The Aikawa
Memorial
Lecture
2022







Program Schedule

7:30 – 8:00am: Arrival / Check-in

8:00 – 8:30am: Continental Breakfast

8:30 – 10:00am: Oral Session I
Moderator: Dr. Brian Gaudette

8:30 Jacob Ingber (Abstract 12)
Dual, but not single, TCR memory T cells mediate costimulatory blockade-resistant rejection of highly ischemic heart allografts

8:45 Elizabeth Woidke (Abstract 48)
C9orf72 controls the balance of Th17 and T regulatory cell fate choice

9:00 Brayden Beathe-Gateley (Abstract 2)
A Mutable HIV Envelope Model Reveals Immune Constraints on Antigen Diversification

9:15 Pavel Nesmiyanov (Abstract 23)
Resident CD8+ T cells regulate bitter taste sensitivity

9:30 Alyssia Broncano (Abstract 6)
Nuclear estrogen receptors modulate regulatory T cell suppressive function

9:45 Katelyn O'Hare (Abstract 25)
Vascular Tissue Expression and Immune Recognition of Citrullinated Self-Proteins in People with HIV

10:00 – 10:30am: Poster Flash Talks
Moderator: Dr. Mike Freeman

10:00 Elizabeth Seidita (Abstract 31)
TGFBRAP1 Overexpression Selectively Inhibits Filovirus and SARS-CoV-2 Entry

10:02 Indrani Das (Abstract 11)
Secretory iRGD-enhanced CAR-T therapy for solid tumors

10:04 Ashomathi Mollin (Abstract 21)
Host-Microbe Interactions Between IgA and Lactobacillus crispatus in the Regulation of Vaginal Homeostasis and Reproductive Health

10:06 Anna Winnicki (Abstract 47)
Identifying Immune Correlates of Protection to Plasmodium vivax using Human Monoclonal Antibodies to Apical Membrane Antigen 1

10:08 Lane Pierson (Abstract 27)
Functional Comparison of Bat STING Orthologs to Understand Innate Immune Modulation in Reservoir Species

10:10 Gracie Carlson (Abstract 8)
Proinflammatory signaling suppresses endothelial ST6Gal1: Interrogating the role of IFN-γ

10:12 Amber Cardani-Boulton (Abstract 7)
CD6 Facilitates Type I Interferon Signaling in T Cells

10:14 Suneha Shelke (Abstract 34)
AI-enhanced workflow for quantification of cell death in the mouse small intestine

10:16 Priya Das Sinha (Abstract 36)
Immune-Metabolic Crosstalk: NO-Driven Regulation of Cytochrome P450

10:18 Taro Banno (Abstract 1)
Interferon Regulatory Factor 4 (IRF4) Promotes Myeloid Cell Differentiation and Function to Mediate Antibody-Mediated Kidney Allograft Rejection in Mice

10:30 – 10:45am: Coffee Break

10:45 – 11:45am: Poster Session I

Abstract #	Presenter	Title
1	**Taro Banno**	Interferon Regulatory Factor 4 (IRF4) Promotes Myeloid Cell Differentiation and Function to Mediate Antibody-Mediated Kidney Allograft Rejection in Mice
4	**Emily Blaum**	The role of ursodeoxycholic acid in mediating antitumor responses in CD19-directed CAR T-cell therapy
7	**Amber Cardani-Boulton**	CD6 Facilitates Type I Interferon Signaling in T Cells
8	**Gracie Carlson**	Proinflammatory signaling suppresses endothelial ST6Gal1: Interrogating the role of IFN-γ
9	**Isaac Chang**	Evaluating the Contribution of Retinoic Acid Receptor (RAR) to HIV-1 Latency in Microglia Cells
10	**Jordan Cress**	WNK1 as a therapeutic target in AML: Synergistic anti-leukemic activity with ATRA
11	**Indrani Das**	Secretory iRGD-enhanced CAR-T therapy for solid tumors

14	**Daniel Kingsley**	Macrophage Integrin Engagement Promotes Pulmonary Metastasis in Osteosarcoma
15	**Kayla Klatt**	Investigating Trypsin 2 as a Regulator of IFN-γ Expression and Downstream Immune Signaling Programs
16	**Bailey Klein**	Mast Cell Derived Histamine Negatively Regulates Hematopoiesis
17	**Hemanta Kumar Datta**	Nuclear Ezrin in B lymphocytes
18	**Katelyn Lemr**	Phenotyping Amyotrophic Lateral Sclerosis patient fecal matter to identify microbes that contribute to neural inflammation
19	**Yuki Maruyama**	Deficiency of Recipient NKG2D or Donor Rae-1 Prevents Acute Antibody-Mediated Rejection in a Mouse Kidney Transplant Model
20	**Sarah McNeer**	Dectin-1 mediates uptake and clearance of a non-temperature adapted clade of *Debaryomyces hansenii*
21	**Ashomathi Mollin**	Host-Microbe Interactions Between IgA and *Lactobacillus crispatus* in the Regulation of Vaginal Homeostasis and Reproductive Health.
22	**Yuta Mukae**	Recipient TLR9 Signaling Is Required for Early Post-Transplant Inflammation Driving Heterologous Donor-Reactive Memory CD8 T Cell Activation in High Risk Allografts
24	**Thu Nguyen**	*TNFRSF13B* mutations enhance macrophages phagocytosis
26	**Steven Overend**	The impact of HMGB1 interaction with RIG-I on detection of viral nucleic acids
32	**Takanori Sekito**	Inhibition of Type I Interferon Signaling Attenuates Acute Antibody-Mediated Rejection Without Altering Early Chronic Alloimmune Injury
42	**Hidetoshi Tsuda**	Recipient Monocytes Are Required for Heterologous Donor-Reactive Memory CD8 T Cell Activation to Reject High Risk Allografts

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

1:00 – 2:30pm: Oral Session II
Moderator: Dr. Stephanie Langel

1:00 Paul Karell (Abstract 13)
Gram-positive targeting antibiotics compromise intestinal humoral immunity

1:15 **Joseph Williams** (Abstract 46)
Co-operation between gasdermin (GSDM) family members, GSDMB and GSDMD, may regulate goblet cell function during homeostasis and is dysregulated during inflammatory bowel disease (IBD)

1:30 **Yingting Zhang** (Abstract 49)
Perturbed neutrophil responses in Irf3-deficient mice protect from CCl4-induced liver fibrosis in mice

1:45 **Ruiting Zhou** (Abstract 51)
Conformation analysis of STAT1 homodimers reveal the immuno-incompetence mechanism in STAT1 GOF patients

2:00 **Hannah Wargo** (Abstract 45)
Microbiota-derived metabolite loss drives Th17-type immune responses in a murine model of Crohn's disease (CD)-like ileitis

2:15 **Saanvi Billakanty** (Abstract 3)
Loss of B cell-intrinsic Ezrin exacerbates airway hyperresponsiveness in allergen-induced asthma

2:30 – 2:45pm: Coffee Break

2:45 – 3:45pm: Poster Session II

Abstract #	Presenter	Title
27	**Lane Pierson**	Functional Comparison of Bat STING Orthologs to Understand Innate Immune Modulation in Reservoir Species
28	**Vanessa Raab**	*TNFRSF13B* influences host immune response to enterobacteria in the mucosa
29	**Michelle Raymond**	Myosin 18A regulates B cell differentiation through restricting the unfolded protein response and TLR7-mediated signaling
30	**Avinaash Kaur Sandhu**	MHC-II trafficking is impaired in Mtb-infected M2-like macrophages that evade CD4+ T-cell recognition
31	**Elizabeth Seidita**	TGFBRAP1 Overexpression Selectively Inhibits Filovirus and SARS-CoV-2 Entry
33	**Ines Selmi**	Condensin Dysregulation Drives Retrotransposable Element Derepression, Senescence, and Intestinal Barrier Loss
34	**Suneha Shelke**	AI-enhanced workflow for quantification of cell death in the mouse small intestine

35	**Sadiq Silbak**	Monoclonal Antibody 5C12 is an Allosteric Inhibitor of Factor XIIa's Active Site
36	**Priya Das Sinha**	Immune-Metabolic Crosstalk: NO-Driven Regulation of Cytochrome P450
37	**Caitlin Snyder**	Macrophage Piezo1 Drives Myofibroblast Differentiation through Cell-Cell Contact
38	**Anish Sriram**	Understanding How Human STING Polymorphisms Modulate SAVI-Variant Functionality
39	**J. Michael Stolley**	Nasal Mucosal Recall Dominance by Recirculating Memory CD8+ T Cells
40	**Vinicius Suzart**	Human memory CD4+ T cells recognize non-infected macrophages bystander to *Mycobacterium tuberculosis* infection
41	**Reyhaneh Tabatabaei**	The effect of altered B cell metabolism on vaccine response in obesity
43	**Kaylynn Vidmar**	Intestinal epithelial cell (IEC)-derived gasdermin C (GSDMC) regulates IL-33 subcellular trafficking during chronic intestinal inflammation
44	**Siyu Wang**	Estrogen Receptor–Dependent Regulation of IgG Glycosylation
47	**Anna Winnicki**	Identifying Immune Correlates of Protection to *Plasmodium vivax* using Human Monoclonal Antibodies to Apical Membrane Antigen 1
50	**Ziyin Zhao**	A Novel Bioinformatic Method to Trace Donor-Specific B Cell Evolution in Transplant Recipients
52	**Bingcheng Wang**	EphA2 receptor tyrosine kinase mediates pan-cancer immune evasion

4:00 – 5:00pm: **Keynote Speaker**
Dr. Craig Maynard
Associate Professor, Department of Pathology, Division of Molecular and Cellular Pathology, Department of Medicine, Division of Gastroenterology and Hepatology, University of Alabama at Birmingham

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

Abstracts

Abstract 1

Interferon Regulatory Factor 4 (IRF4) Promotes Myeloid Cell Differentiation and Function to Mediate Antibody-Mediated Kidney Allograft Rejection in Mice

Taro Banno, Danielle D. Kish, Nina Dvorina, William M. Baldwin, Robert L. Fairchild

Cleveland Clinic Lerner Research Institute, Cleveland, OH, USA

Antibody-mediated rejection (ABMR) is a major cause of kidney allograft loss. In B6.CCR5-/- recipients of fully MHC-mismatched A/J kidneys, high donor-specific antibody (DSA) titers and NK cell activation mediate acute graft injury between days 18–25. Myeloid cells producing myeloperoxidase (MPO) enhance monocyte/macrophage function and NK activation, whereas MPO deficiency shifts injury toward chronic ABMR with fibrosis and autoantibody production. During acute ABMR, infiltrating monocytes express high levels of interferon regulatory factor 4 (IRF4), while macrophages express low levels. In contrast, during chronic ABMR, macrophages express high IRF4 and monocytes low IRF4. To define the role of IRF4 in myeloid cells, we generated B6.CCR5-/-LysMCreIRF4fl/fl mice and used them as recipients of A/J kidneys. While allografts in B6.CCR5-/- recipients were acutely rejected, 83% of allografts survived beyond day 80 in IRF4-deficient recipients. Despite persistently high DSA and autoantibody levels, NK cell and Ly6Chi monocyte infiltration was markedly reduced. Histological analyses at day 90 showed minimal fibrosis and mild cellular infiltration, with no evidence of chronic ABMR. These findings demonstrate that IRF4 is required for myeloid cell-mediated effector functions driving both acute and chronic ABMR, independent of circulating antibody levels.

Abstract 2

A Mutable HIV Envelope Model Reveals Immune Constraints on Antigen Diversification

Brayden Beathe-Gateley[1], Hui. Liu[2], Mayara Garcia De Mattos Barbosa[1], Yotam Bar-On[3], Tracy Pasieka[3], Jeffrey L. Platt[1], Marilia Cascalho[1]

[1] Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH. [2] Department of Immunology, University of Michigan, Ann Arbor, MI. [3] Technion University, Haifa, Israel

HIV persistence and vaccine failure are driven in part by rapid viral diversification, which enables immune evasion. Activated B cells likewise generate rapid sequence diversity through somatic hypermutation. We leveraged this parallel to create an Env-mutator model in which HIV-1 envelope diversification is driven by the B-cell mutational machinery. We engineered a murine "mutable transgene" model in which an HIV-1 subtype B envelope (*Env*) transgene is conditionally expressed under the murine Ig λ light-chain promoter and Ig heavy-chain intronic enhancer, which diversifies through B-cell somatic hypermutation. Following NP-OVA immunization, activated B cells secrete Env, and the Env insert accumulated mutations in germinal center B cells at frequencies comparable to those of immunoglobulin genes. Many non-synonymous mutations matched variants observed in naturally circulating HIV Env sequences, indicating that this system reproduces features of viral diversification seen in infection. However, diversification was not unrestricted. Env-producing cells declined after repeated immunization, consistent with immune-mediated selection against variant-expressing cells in a CD8 T cell–dependent manner. This constraint was relieved by immunosuppression and by CD8 T-cell depletion, which increased Env mutation frequency and expanded the persistence of mutations predicted to generate high-affinity MHC class I-binding peptides. Together, these findings identify CD8-dependent immune pressure as a major force shaping evolving Env repertoires in vivo. This model provides a tractable platform for studying how adaptive immunity constrains antigen diversification and suggests a framework for vaccine strategies designed to anticipate viral evolution rather than chase it.

Abstract 3

Loss of B cell-intrinsic Ezrin exacerbates airway hyperresponsiveness in allergen-induced asthma

Saanvi Billakanty, Alayna Stroberg, Kewal Asosingh, and Neetu Gupta

Department of Inflammation and Immunity, Cleveland Clinic Research, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH

Asthma accounts for substantial healthcare burden around the world. Inflammation, impaired lung function, and airway remodeling are key correlates of pathogenesis in allergen-induced asthma. However, despite many advancements, the molecular regulation of each of these processes is still poorly understood. Ezrin, a plasma membrane actin cytoskeleton linker protein, is expressed in immune and non-immune cells of the airways. We previously reported that Ezrin negatively regulates humoral immunity, suggesting that it may also influence B cell function in the context of asthma. Here, we show that upon sensitization and challenge with house dust mite (HDM) allergens, mice with B cell-specific deficiency of Ezrin (Ez-def) display significantly increased airway hyperresponsiveness (AHR) – increased stiffness, decreased compliance, and lower airflow – compared to control $Mb1^{cre/+}$ mice. No significant differences were observed in leukocyte infiltration in the bronchoalveolar lavage fluid (BALF) of Ez-def mice. While cytokine levels in serum and BALF were not altered by Ezrin deletion, pro-inflammatory cytokines VEGF, IL-12 p40, and LIX were significantly higher and Eotaxin, IL-1β, and IP-10 were lower. Total and HDM-specific IgE levels in BALF and serum trended upwards, and there were significantly more B cells among Ez-def mice. Lung tissues in Ez-def mice had no changes in fibrosis but increased mucus production and deposition of Substance P, a neurotransmitter that induces smooth muscle contractility and can thus worsen AHR. Taken together, our data indicate that B cell-intrinsic Ezrin limits development of AHR during allergic asthma, potentially by regulating local interactions of B cells with structural cells of the lungs.

Abstract 4

The role of ursodeoxycholic acid in mediating antitumor responses in CD19-directed CAR T-cell therapy

Emily Blaum[1,2] Manishkumar S. Patel[1], Akansha Jalota[1], Ina Nemet[3], Brian T. Hill[4], and Neetu Gupta[1]

[1]Department of Inflammation and Immunity, Cleveland Clinic Research
[2]Medical Scientist Training Program, Case Western Reserve University
[3]Department of Heart, Blood, and Kidney Research, Cleveland Clinic Research
[4]Hematology and Medical Oncology, Taussig Cancer Institute, Cleveland Clinic

CD19-directed chimeric antigen receptor (CAR) T-cell therapy has revolutionized treatment for relapsed/refractory large B-cell lymphoma (r/r LBCL). However, many patients experience incomplete or non-durable responses, highlighting an unmet need to enhance CAR T-cell effectiveness and persistence. Growing evidence indicates that the phenotypic states of CAR T-cells at the time of infusion and during *in vivo* expansion, particularly stem-like and T_H2 cells, are critical factors for persistence and antitumor activity. Supporting the development and maintenance of these T-cell phenotypes during manufacturing or after infusion may help address this clinical need. Once infused, CAR T-cells encounter a complex metabolic environment, including host and microbiome-derived metabolites that can influence their function. Secondary bile acids, synthesized from primary bile acids by microbes in the large intestine, are cholesterol-derived metabolites. Among these, ursodeoxycholic acid (UDCA) has been shown to restrain T_{reg} differentiation and synergize with anti-PD-1 therapy, indicating a direct influence on T-cell-mediated anti-tumor responses. This study examines UDCA's impact on CAR T-cell function. Analysis of plasma from 80 r/r LBCL patients demonstrates that secondary bile acids are abundant in circulation on the day of apheresis when T-cells are collected for manufacturing. Using an *in vitro* approach, I show that UDCA enhances TCR-mediated T-cell activation but dampens IFNγ secretion, a cytokine known to restrict antitumor responses by inhibiting stem-like T-cells. Overall, these findings support a model in which UDCA modulates T-cell activation and differentiation to potentially promote durable antitumor responses.

Abstract 5

withdrawn

Abstract 6

Nuclear estrogen receptors modulate regulatory T cell suppressive function

Alyssia V. Broncano[1], Sarah M. Stark[1], Wendy A. Goodman[1]

[1]Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH

Autoimmune diseases are driven by intricate combinations of genetic and non-genetic factors, but the specific mechanisms underlying these disorders are not fully understood. 17β-estradiol (estrogen, E2) is a steroid sex hormone with potent immunoregulatory functions that contributes to autoimmunity. E2 signaling through the nuclear estrogen receptors alpha and beta (ERα and ERβ) have clear immunomodulatory effects, with ERα generally promoting and ERβ generally restraining inflammation. Previous work from our lab and others demonstrated that ERβ-specific signaling is reduced in inflamed mucosal tissues and T cells from female Crohn's disease patients, supporting the premise that ERβ negatively regulates inflammation. Our current study investigates the functional roles of ERβ in regulatory T cells (Tregs), in which we hypothesized that ERβ promotes Treg suppressive function. To test this, we performed standard *ex vivo* Treg suppression assays using wild-type (WT), ERα-KO, or ERβ-KO Tregs with or without E2 treatment. Our results show that E2 reduces *ex vivo* WT and ERβ-KO Treg suppressive function. We also tested Treg suppression *in vivo* using a T cell transfer colitis model. Immunodeficient Rag2-KO mice were co-injected with WT effector T cells (CD4+CD25-) and WT, ERα-KO, or ERβ-KO Tregs (CD4+CD25+) to test the ability of Tregs to prevent colitis development. We found that recipients of ERβ-KO Tregs display more severe disease compared to recipients of WT Tregs, including stunted weight gain and increased histological inflammation. These findings show that deletion of ERβ impairs Treg suppression, thus supporting a critical role for ERβ in promoting Treg function.

Abstract 7

CD6 Facilitates Type I Interferon Signaling in T Cells

Amber Cardani-Boulton[1,2], Adam M Boulton[3], Ruiting Zhou[3], Feng Lin, Yuxin Wang[1,3], and Cornelia C Bergmann

[1]Cleveland Clinic Lerner College of Medicine of Case Western Reserve University School of Medicine, Cleveland, OH, USA
[2]Department of Microbial Sciences in Health, Cleveland Clinic Research, Cleveland, OH, USA
[3]Department of Immunity and Inflammation, Cleveland Clinic Research, Cleveland Clinic, Cleveland, OH

CD6 is a clinically targeted scavenger receptor almost exclusively expressed on T cells that is established to incorporate into the T cell receptor signalosome, where it can both positively and negatively modulate T cell activation in a context dependent manner. However, the mechanisms underlying the diverse effects of CD6 on T cell activation and differentiation have yet to be elucidated. Our studies have identified a novel immune-regulatory role for CD6, whereby CD6 enables type I IFN signaling in both human and murine T cells. Genetic deletion or blocking CD6 prevented type I interferon induced STAT1 phosphorylation, resulting in limited expression of type I interferon stimulated genes. Mechanistic studies revealed that CD6 interacts with the IFNalpha receptor complex extracellularly as well as intracellularly where it also binds to STAT1. The loss of type I IFN signaling through STAT1/STAT2 in the absence of CD6 resulted in the upregulation of antiapoptotic proteins and promoted CD4 T follicular helper cell differentiation, while simultaneously preventing upregulation of cytotoxic effectors. Overall, these data establish that CD6 permits canonical STAT1/STAT2 type I IFN signaling in murine and human CD4 and CD8 T cells, thus designating CD6 as a strong therapeutic candidate for modulating type I IFN-induced T cell effector functions.

Abstract 8

Proinflammatory signaling suppresses endothelial ST6Gal1: Interrogating the role of IFN-γ

Gracie C. Carlson[1], Siyu Wang[1], Leandre M. Glendenning[1], Austin D. Silva[2], Susan L. Bellis[2], Brian A. Cobb[1]

[1]*Department of Pathology, Case Western Reserve University School of Medicine*
[2]*Department of Cell Developmental and Integrative Biology, University of Alabama at Birmingham*

IgG is a critical component of the humoral immune response and is integral to immunologic homeostasis. Glycan composition at the conserved N-glycosylation N297 site within the Fc CH2 domain alters the effector function of IgG by skewing Fc receptor binding. Terminal α2,6 sialylation of the Fc glycan by the sialyltransferase ST6Gal1 has been shown to decrease the ability of an antibody to elicit a proinflammatory effector function. This is supported by 4 decades of epidemiologic data linking decreased IgG terminal sialylation and galactosylation with inflammation and the severity of inflammatory diseases. Recently, we published our findings, showing that endothelial cells can sialylate IgG *in vitro* and that loss of the FcRn-mediated recycling pathway results in a significant reduction in IgG sialylation *in vivo*. However, little is known about the regulation of ST6Gal1 in the endothelium, and thus the role of the vasculature in regulating circulating IgG sialylation levels remains unclear. We report here that the inflammatory stimuli IFN-γ and LPS decrease endothelial ST6Gal1 and that the cells' ability to sialylate IgG in culture is also reduced. The current goals of this project focus on interrogating the signaling mechanisms that allow IFN-γ to regulate endothelial ST6Gal1 expression and the cells' ability to sialylate IgG.

Abstract 9

Evaluating the Contribution of Retinoic Acid Receptor (RAR) to HIV-1 Latency in Microglia Cells

Isaac Chang, Jonathan Karn, Yoelvis Garcia-Mesa

Molecular Biology and Microbiology, School of Medicine, Case Western Reserve University, Cleveland, Ohio, USA

Background: RAR activation suppresses the NF-κB signaling pathway, resulting in the reduced expression of key proinflammatory cytokines, specifically TNF-α, IL-1β, and IL-6. Consequently, we hypothesize that RAR agonists may function as HIV-latency promoting agents in microglial cells, the primary HIV-1 reservoir in the brain.
Methods: To probe our hypothesis, we first treated the HIV-latently-infected microglia cell line HC69 with increasing concentrations of adapalene (RAR agonist, from 60 to 500 nM). Data collected from the HC69 cells were reproduced using iPSC-derived microglia (iMG) infected with HIV-1 single-round (iMG/HIV) or with the HIV macrophage tropic virus AD8, in the presence of iPSC-derived cortical neurons (iCORT, cocultures) or iCORT and iA (iPSC-astrocytes, tricultures). Cells were analyzed by flow-cytometry, RT-qPCR and microscopy. Statistical analyses were done using Student's t-test.
Results: Adapalene, potent RAR agonist, significantly reduced HIV expression in HC69 cells, by ~40% detected via flow-cytometry and up to ~80% via RT-qPCR. Staining of adapalene-treated HC69 cells demonstrated inhibited p65 nuclear translocation compared to untreated controls. iCORT+MG/HIV cocultures treated with 50 nM adapalene also showed ~70% HIV suppression. Finally, HIV-1-exposed cocultures and tricultures treated with adapalene revealed viral expression reductions of ~60% and ~50%, respectively.
Conclusion: Data from this study demonstrates that adapalene, an RAR agonist, can silence HIV-1 expression in microglia. Although additional research is required to fully validate these effects, our results suggest a novel therapeutic pathway aimed at improving the long-term health and well-being of HIV-infected patients.

Abstract 10

WNK1 as a therapeutic target in AML: Synergistic anti-leukemic activity with ATRA

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Impaired differentiation is a key pathological feature of Acute Myeloid Leukemia (AML). AML differentiation-inducing agents such as all-trans retinoic acid (ATRA) have shown great promise to treat AML patients as seen by improved overall survival and relapse rates. However, this success has been limited to a subset of AML patients. This highlights the need to identify more differentiation therapy options that could be effective for other AML subtypes. In this study, we identified With-no-Lysine(K) kinase 1 (WNK1) as a novel regulator of AML differentiation arrest. We show evidence of WNK1 dysregulation in AML patients demonstrated by its increased expression compared to healthy controls. Inhibiting WNK1 induced granulocytic differentiation of AML cell lines and patient samples. Inducing AML differentiation through WNK1 inhibition also coincided with decreased growth and survival. Mechanistically, we show that WNK1 inhibition induces differentiation by promoting MEK-ERK activity and increasing C/EBPβ activity and expression, resulting in increased transcription of myeloid differentiation genes. Finally, we illustrate that combining WNK1 inhibition with ATRA further boosts ATRA's efficacy, synergistically increasing differentiation. C/EBPβ expression was also enhanced by this combination treatment, further supporting its role for C/EBPβ in mediating WNK1-inhibition induced AML differentiation. Taken together, our findings suggest that WNK1 negatively regulates ERK phosphorylation and activity. Inhibiting WNK1 releases ERK suppression, increasing C/EBPβ activity and expression which leads to myeloid differentiation. Overall, our findings reveal a novel role for WNK1 in promoting differentiation arrest and highlight its potential as a new therapeutic target for AML treatment.

Abstract 11

Secretory iRGD-enhanced CAR-T therapy for solid tumors

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Chimeric Antigen Receptor T-cell (CAR-T) therapy is a form of cancer immunotherapy that engineers T cells to recognize a specific tumor antigen and lyse the corresponding tumor cell. CAR-T therapy is rapidly evolving and has revolutionized the treatment of certain hematological malignancies. Its use against solid cancers such as pancreatic cancer, however, has limited success, largely due to the impenetrability of the immunosuppressive tumor microenvironment (TME). iRGD is a cyclic tumor-penetrating peptide that binds to αvβ3 and αvβ5 integrins, leading to its proteolytic cleavage and release of the C-terminal, RGD-containing CendR motif. Binding of the CendR motif to the cellular transport protein neuropilin-1 (NRP-1) triggers an increase in transcytosis, ECM remodeling, and vascular permeability, thus facilitating entry of therapeutic agents into the tumor. As such, iRGD has been used to increase tumor penetration and efficacy of chemo-, nano-, and immunotherapies in preclinical and clinical studies. Here, we demonstrate the unprecedented combination of iRGD with CAR-T therapy. We engineer B7H3 CAR-T cells to secrete iRGD and demonstrate their *in vitro* efficacy against pancreatic cancer cells. Preliminary data demonstrate an increase in iRGD CAR-T infiltration into PDAC tumor models *in vivo*. Data also suggest a potential role for iRGD alone in inhibiting TGFβ activation, as well as reducing angiogenesis and stromal fibers in the pancreatic cancer TME. Our study, if successful, will contribute an innovative and translatable advancement to the treatment of solid tumors with CAR-T therapy.

Abstract 12

Dual, but not single, TCR memory T cells mediate costimulatory blockade-resistant rejection of highly ischemic heart allografts

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The risk of poor clinical transplant outcomes is increased by prolonged allograft cold ischemic storage (CIS) prior to transplant and the pre-transplant presence of circulating donor-reactive memory T cells. In unsensitized recipients, a proportion of memory T cells generated by infection and other encountered antigens are cross-reactive with allogeneic MHC molecules, so-called heterologous immunity. Prolonged CIS of heart allografts leads to increased ischemia-reperfusion injury (IRI) and activation of heterologous donor-reactive memory T cells to mediate CTLA-4Ig-resistant rejection. T cells expressing two unique T cell receptors composed of a single β chain paired with two different α chains constitute 15-20% of the peripheral T cell repertoire and are important mediators of graft-versus-host disease, but their role in solid organ allograft injury and rejection is unknown.

Single TCR mice were generated by crossing wildtype B6 with B6.Cα$^{-/-}$ mice to generate B6.Cα$^{+/-}$ progeny. A/J hearts underwent 0.5hr or 8hr CIS prior to transplantation to B6 or B6.Cα$^{+/-}$ recipients conditioned with 250μg CTLA-4Ig or control rat IgG (i.p., d0,+1). Allografts were harvested at rejection or on day 2 post-transplant for analysis.

Recipients of long CIS allografts unable to produce dual TCR T cells are more sensitive to CTLA-4Ig treatment, having prolonged allograft survival (MST 33 vs 17.5 days). Without CTLA-4Ig, wildtype and single TCR recipients reject allografts at similar timepoints due to naïve donor-reactive T cell activation. These findings demonstrate that donor-reactive memory CD8^{+} T cells with single TCR cannot mediate CTLA-4Ig-resistant rejection of highly ischemic allografts, providing novel insights into cellular rejection mechanisms.

Abstract 13

Gram-positive targeting antibiotics compromise intestinal humoral immunity

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Antibiotics are ubiquitous, life-saving medicines used across the globe, but intestinal infection secondary to their use results in thousands of deaths annually in the United States alone. Mirroring this clinical observation, antibiotics greatly improve the ability of *Salmonella* spp. to establish infection in the mouse colon. We found that antibiotics with coverage against gram-positive microbes were particularly potent in eliciting severe infection even in vaccinated mice that secrete large quantities of neutralizing, *Salmonella*-specific IgA. To investigate the mechanism by which certain antibiotics increase the risk of intestinal infection, we assessed the impact of antibiotics of varying coverages on fecal IgA levels. We identified antibiotics most associated with intestinal infection to precipitously reduce luminal IgA concentrations in the lower intestine just 48hrs after oral or intraperitoneal administration. Mechanistically, elimination of gram-positive microbes results in carbohydrate accumulation in the lower intestine preventing efficient water absorption at this site, thereby diluting pathogen-specific IgA. Importantly, this occurs independent of any direct effect on IgA secreting or transporting cells, thus unveiling defects in luminal physiology as a previously unappreciated underpinning of IgA deficiency. We are now investigating oral IgA supplementation as a potential prophylactic in this model, a strategy that may translate to reduced incidence of antibiotic associated intestinal infection in the future.

Abstract 14

Macrophage Integrin Engagement Promotes Pulmonary Metastasis in Osteosarcoma

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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 (BMDMs) 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 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 15

Investigating Trypsin 2 as a Regulator of IFN-γ Expression and Downstream Immune Signaling Programs

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Interferon gamma (IFN-γ) is a central regulator of immune responses that coordinates host defense against infection and cancer, yet the upstream pathways governing its expression across immune cell subsets remain incompletely understood. While trypsin 2 is classically studied as a digestive serine protease, emerging evidence suggests it may play a role in immune regulation. To define the immunologic function of trypsin 2, we generated a global trypsin 2 knockout mouse model and performed RNA sequencing on splenocytes under basal and IL-2-stimulated conditions. Transcriptomic analysis revealed broad dysregulation of immune-related pathways in the absence of trypsin 2, with the most pronounced differences observed following IL-2 stimulation. Notably, cytokine and chemokine signaling networks were significantly altered, including reduced expression of *Ifng* and downstream interferon-responsive genes. At the protein level, flow cytometry and ELISA confirmed a significant reduction in IFN-γ production in trypsin 2 knockout splenocytes following cytokine stimulation. This defect was observed across multiple lymphocyte subsets, including $CD4^+$ T cells, $CD8^+$ T cells, and natural killer (NK) cells. Consistent with these findings, multiplex cytokine analysis demonstrated decreased levels of IFN-γ–associated chemokines such as CXCL10 and increased expression of inflammatory cytokines including IL-17A in splenocyte conditioned media, supporting a shift in immune signaling programs. Together, these data identify trypsin 2 as a previously unrecognized regulator of IFN-γ expression and immune signaling networks. These findings provide a foundation for understanding how trypsins influence immune function and highlight trypsin 2 as a potential modulator of immune responses in inflammatory disease and cancer.

Abstract 16

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 17

Nuclear Ezrin in B lymphocytes

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Ezrin is a plasma membrane–actin cytoskeleton crosslinking protein that plays an important role in B cell function, including antigen receptor signaling, proliferation, and differentiation. Previously, we showed that deletion of Ezrin in B cells enhances signaling responses, increases plasma cell differentiation, and alters immune responses. Although Ezrin is traditionally associated with the cell membrane and known to be a cytoskeletal protein, recent findings suggest it may also localize to the nucleus of non-immune cells.

In this study, we investigated whether Ezrin is present in the B cell nucleus, how its localization is regulated, and its potential nuclear functions. Using high-resolution imaging and biochemical fractionation, we demonstrate that Ezrin is indeed present in the nucleus of B cells. Nuclear localization depends on three conserved nuclear localization sequences, as deletion of these sequences significantly reduces its nuclear presence. Additionally, Ezrin interacts with nuclear transport proteins, including importin and exportin, indicating active regulation of its nuclear trafficking. Notably, stimulation of the B cell antigen receptor decreases Ezrin localization in the nucleus.

These findings establish that Ezrin is a nucleocytoplasmic protein in B cells and that its localization is dynamically regulated. Ongoing studies aim to determine the functional role of nuclear Ezrin, particularly in processes such as gene expression, signal transduction, activation, proliferation, differentiation, and humoral immune responses.

Abstract 18

Phenotyping Amyotrophic Lateral Sclerosis patient fecal matter to identify microbes that contribute to neural inflammation

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A G_4C_2 hexanucleotide repeat expansion in the *C9ORF72* gene is the most common cause of Amyotrophic lateral sclerosis. Loss of *C9orf72* function in mice leads to systemic and neural inflammatory and autoimmune phenotypes that model inflammatory phenotypes observed in patients. In environments that harbor gut microbes with greater innate immune stimulatory potential, *C9orf72*$^{-/-}$ mice develop inflammatory neural phenotypes leading to premature death, while in environments with relatively benign gut commensals or when benign commensal are introduced by fecal microbial transplantation these mice experience less inflammation and live a normal lifespan. We hypothesize that ALS patients with a rapid disease course harbor gut microbes that can enhance systemic and neural inflammation. To test this, bacteria was isolated from ALS patient fecal samples and healthy controls and applied to bone marrow derived macrophages to evaluate innate immune stimulatory potential. We observed bacteria derived from fast-progressing males has the highest stimulatory potential. We then reconstituted germ-free *C9orf72*$^{+/+}$, *C9orf72*$^{+/-}$, and *C9orf72*$^{-/-}$ mice for 3-months with either fast- or slow-progressing ALS patient, measured the extent of systemic and neural inflammation compared to germ-free control mice. We have isolated candidate probiotics from healthy human fecal matter and have demonstrated the ability of the probiotics to suppress the inflammatory stimulatory potential of our human fecal matter in reconstituted GF *C9orf72*$^{+/-}$ mice. Our work provides insight into the physiologic signals that govern immunologic tolerance to gut microbes in ALS and establish a pre-clinical platform to evaluate and therapeutically perturb the neural inflammatory potential of each patient's gut microbiome.

Abstract 19

Deficiency of Recipient NKG2D or Donor Rae-1 Prevents Acute Antibody-Mediated Rejection in a Mouse Kidney Transplant Model

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Acute-antibody-mediated rejection (aABMR) is a major cause of kidney graft failure. We previously reported that B6.CCR5$^{-/-}$ recipients of kidney allografts reject complete MHC-mismatched kidney allografts between days 18–28 post-transplant whereas these allograft survive long-term in wild-type C57BL/6 recipients. This rejection in B6.CCR5$^{-/-}$ recipients requires both DSA-production and NK cell activation within the allograft. In this study we investigated mechanisms underlying NK cell activation during aABMR.

Wild-type A/J or A/J.Rae-1$^{-/-}$ kidneys were transplanted into B6.CCR5$^{-/-}$ recipients; and, A/J kidneys were transplanted into either B6.CCR5$^{-/-}$NKG2D$^{-/-}$ recipients or B6.CCR5$^{-/-}$ recipients treated with anti-NKG2D-mAb.

In B6.CCR5$^{-/-}$ recipients, DSA titers peaked on day15 post-transplant, accompanied by a parallel increase in mRNA levels of Rae-1e, a ligand for NKG2D. While kidney allografts were rejected between days 18-28 in B6.CCR5$^{-/-}$ recipients, A/J.Rae-1$^{-/-}$ kidneys exhibited long-term survival in the same recipients. Similar prolonged graft survival was observed following transplantation of A/J kidneys to B6.CCR5$^{-/-}$NKG2D$^{-/-}$ recipients or treatment with anti-NKG2D mAb. Flow cytometric analysis in allografts on day15 post-transplant revealed that when compared with NK cells infiltrating A/J allografts in B6.CCR5$^{-/-}$ recipients, either recipient NKG2D deficiency or donor graft Rae-1 deficiency reduced NK cell accumulation, proliferation, and NK cell expression of CD107a^{+}. In addition, flow cytometry demonstrated reduced numbers of inflammatory Ly6C^{+} monocytes within Rae-1-deficient allografts and A/J-allografts from B6.CCR5$^{-/-}$NKG2D$^{-/-}$ recipients. Moreover, qPCR analysis of allografts showed that Rae-1e expression was low-absent in Rae-1-deficient donor allografts and NKG2D-deficient recipients. These findings suggest that interference with recipient NK cell NKG2D or its ligand Rae-1 within graft inhibits aABMR and prolongs graft survival despite persistently high DSA levels.

Abstract 20

Dectin-1 mediates uptake and clearance of a non-temperature adapted clade of *Debaryomyces hansenii*

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Debaryomyces hansenii is a food yeast that is widely recognized as safe. However, our lab found that *D. hansenii* can be isolated from the ulcers of Crohn's disease (CD) patients and delay intestinal wound healing in murine models of intestinal injury. Establishment of a strain collection of isolates derived from food or patient sources revealed that the majority of food strains segregated into a distinct clade marked by the inability to grow at 37C. Despite being non-viable in the host, we found that serum antibody from IBD patients bound strongly to food strain of *D. hansenii*, leading us to hypothesize that food strains of *D. hansenii* can activate immune responses during inflammation and barrier breach.

To decipher how food strains are first sensed by the immune system, we utilized bone-marrow derived macrophages (BMDMs) and demonstrated that BMDMs phagocytose non-viable food strains, mediated by the receptors Dectin-1 and CR3. Phagocytosis resulted in secretion of TNF and IL-6, especially in BMDMs primed with LPS and IFNγ. In a mouse model of DSS-induced colitis, administration of food strains to mice deficient in Dectin-1 exacerbated disease, resulting in more severe weight loss and formation of deep ulcers. Together, this data supports that even non-viable food strains can activate immune cells. Future studies aim to decipher the role of Dectin-1 in controlling the immune response to food strains of *D. hansenii*. These findings will have significant implications for the safety of these strains in vulnerable populations and their use in the food supply.

Abstract 21

Host-Microbe Interactions Between IgA and *Lactobacillus crispatus* in the Regulation of Vaginal Homeostasis and Reproductive Health.

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Under homeostatic conditions, the vaginal epithelium is typically colonized by a *Lactobacillus*-dominant (LD) microbiota, particularly with *Lactobacillus crispatus,* a commensal that promotes an anti-inflammatory environment and limits pathogenic colonization. A microbial shift towards a non-LD microbiota can lead to bacterial vaginosis, a prevalent urogenital condition associated with mucosal inflammation, disruption to the epithelium, and adverse reproductive outcomes. Despite its high prevalence, the host-microbe interactions that maintain a LD microbiota remain poorly understood. Recent evidence implicates immunoglobulin A (IgA) in maintaining optimal vaginal health. We hypothesize that IgA binding modulates the structural, physiological, and metabolic properties of *L. crispatus*, enhancing vaginal persistence and epithelial adherence within the female reproductive tract (FRT). Using flow cytometry, IgA- and IgG-bound bacteria isolated from cervicovaginal swabs, collected in a comprehensive observational cohort (THRIVE), were quantified and the IgA-bound and –unbound bacterial fractions were sorted for 16S rRNA sequencing. An electrochemiluminescence assay and spectral flow cytometry were used to assess antibody binding to *L. crispatus* ex vivo. RNA-seq was performed to evaluate bacterial transcriptomic changes associated with antibody binding. We found that most vaginal microbes are antibody-bound, with IgA predominating over IgG. *L. crispatus* was enriched in the IgA-bound fraction and IgA demonstrated a higher binding capacity. IgA upregulated genes associated with pilus formation and prophages. Our findings provide a basis for investigating how IgA regulates *L. crispatus* structure and physiology. Future studies will combine adherence and competition assays with molecular analyses of glycan-dependent interactions to define host-microbe interactions at the epithelial surfaces of the FRT.

Abstract 22

Recipient TLR9 Signaling Is Required for Early Post-Transplant Inflammation Driving Heterologous Donor-Reactive Memory CD8 T Cell Activation in High Risk Allografts

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We previously reported that increasing duration of cold ischemic storage (CIS) in mice enhances early infiltration of innate and memory T cells into the graft and p40 homodimer (HD) induced heterologous donor-reactive memory CD8 T cell proliferation and expression of effector functions mediating CTLA-4Ig-resistant rejection of the high-risk allografts. Administration of exogenous p40 HD induces memory CD8 T cell proliferation within low ischemic allografts but not their expression of effector functions. Based on these findings, we hypothesized that the increased ischemic environment in the allografts provides mechanistic components provoking the memory CD8 T cells to both proliferate and express effector functions and that a pathogen recognition receptor signaling is likely to drive this increased intragraft alloimmune response. Supporting this, we found that mitochondrial DNA, a known TLR9 ligand, was markedly elevated in serum of high-risk allograft recipients 24 hours post-transplant, suggesting increased activation of TLR9 signaling by prolonged CIS. To test this, we transplanted complete MHC mismatched A/J heart allografts subjected to 8 hrs CIS into wild type or TLR9-/- B6 mice. High ischemic allografts in B6.TLR9 deficient recipients had marked decreases in macrophage and Ly6C+ monocyte infiltration and memory CD4 and CD8 T cell proliferation on day 2 post-transplant with extended survival in CTLA-4Ig conditioned recipients. These results indicate that recipient cells infiltrating high ischemic allografts are activated through TLR9 signaling to provide factors increasing early post-transplant inflammation that, in turn, stimulate donor-reactive memory CD8 T cell proliferation and effector functions mediating acute graft injury and CTLA-4Ig resistant rejection.

Abstract 23

Resident CD8+ T cells regulate bitter taste sensitivity

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Tissue-resident memory $CD8^+$ T cells ($CD8^+$ TRM) serve as long-lived immune sentinels at barrier surfaces, where they enable rapid local responses to pathogen invasion. While $CD8^+$ TRM populations have been well characterized in organs such as the lung and intestine, far less is known about their development and function in the oral mucosa. To investigate this, we established an experimental system that robustly generates antigen-specific $CD8^+$ TRM within the oral tissues of specific pathogen–free mice.

Using this model, we found that oral $CD8^+$ TRM preferentially accumulate within taste papillae. Targeted reactivation of these cells using viral peptide stimulation induced pronounced inflammation in taste tissues and, in some cases, direct penetration of $CD8^+$ TRM into taste buds, indicating that these structures may represent vulnerable sites for viral exposure. These observations led us to propose that immune recall activity by oral $CD8^+$ TRM could influence gustatory function.

To explore this possibility, bulk RNA sequencing was performed on circumvallate and soft palate taste tissues harvested 12 hours after $CD8^+$ TRM reactivation. This analysis revealed marked induction of multiple bitter taste receptor (Tas2r) genes. Correspondingly, behavioral assays demonstrated that mice with reactivated oral $CD8^+$ TRM exhibited heightened aversion to the bitter compound quinine. Importantly, this effect was abolished when $CD8^+$ TRM were depleted prior to peptide challenge.

Together, these findings uncover a previously unappreciated role for oral $CD8^+$ TRM in regulating bitter taste perception and reveal a mechanism by which adaptive immune responses can directly alter sensory experience.

Abstract 24

TNFRSF13B mutations enhance macrophages phagocytosis

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TNFRSF13B encodes TACI (Transmembrane Activator and CAML Interactor), a receptor expressed primarily by B and T lymphocytes. Its ligands, BAFF and APRIL, are mainly produced by myeloid cells and exist in both membrane-bound and soluble forms. Emerging evidence suggests that TACI is also expressed by myeloid cells and contributes to host defense against *Leishmania major* in murine models; however, its role in myeloid cell function remains poorly understood. In this study, we investigate how common TACI variants influence macrophage function, including antibody-dependent and -independent phagocytosis of bacterial pathogens and macrophage polarization, using murine models and human cell culture systems. We hypothesize TACI expression in macrophages is mediated by NFκB, and that macrophage-expressed TACI variants enhance phagocytic capacity through both antibody-dependent and independent mechanisms. Furthermore, we propose that these effects may be mediated by altered post-translational modifications of the Fc domain of immunoglobulins in mice harboring TACI variants compared to wild-type controls. Preliminary data from mice expressing monoallelic or biallelic forms of the common TACI variant A144E (homologous to the human A181E variant) demonstrate that peritoneal macrophages exhibit increased uptake of GFP-expressing gram-negative bacteria relative to wild-type macrophages. These findings suggest that TACI variants may enhance macrophage inflammatory function and promote more efficient clearance of pathogens that rely on phagocytosis for elimination.

Abstract 25

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

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People with HIV (PWH) 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, 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. We have found that PWH on ART were more likely than PWoH to have anti-citrullinated protein antibodies, which have been linked to increased CVD risk among the general population, and more likely to have citrullinated-protein reactive CD8+ T cells compared to PWoH. Using immunohistochemistry, we can detect citrullinated proteins within arterial tissue that may serve as antigenic targets for these antibodies and for CD8+ T cells. We have found that internal carotid artery tissues from PWH have more citrullinated proteins than carotid atherosclerotic plaques from PWoH and that higher levels of citrullinated proteins present within arterial tissues are correlated to worse histological scores of atherosclerosis. Using single-cell RNA sequencing from atherosclerotic plaques of PWoH, we can detect macrophages, mast cells, and plasmacytoid dendritic cells that express PADI2 or PADI4, enzymes that catalyze protein citrullination, although there is no clear signaling pathway driving their expression based on differential gene expression. Taken together, our findings indicate that protein citrullination by myeloid cells within arterial tissues may be a possible mechanism contributing to increased CVD risk among PWH.

Abstract 26

The impact of HMGB1 interaction with RIG-I on detection of viral nucleic acids

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Retinoic acid-Inducible Gene I (RIG-I) is a pattern recognition receptor that senses foreign nucleic acids in the cytosol. RIG-I activation triggers the mitochondrial antiviral signaling (MAVS) cascade and leads to type I interferon production. High Mobility Group Box 1 (HMGB1) is a non-histone DNA binding protein that resides in the nucleus of unchallenged cells. Exposure of cells to microbial associated pattern molecules will trigger translocation of nuclear HMGB1 to the cytoplasm where it regulates autophagy and is consequently released into the extracellular space where it acts as a danger signal. In viral infection, cytosolic HMGB1 is thought to act as a nucleic acid chaperone and enhance detection of viral nucleic acids, although direct HMGB1 interaction with nucleic acid sensors such as RIG-I has not been reported.

Our group recently discovered that HMGB1 binds to a small amino acid motif (target of HMGB1; ToH1) to regulate target protein functions. Bioinformatic modeling demonstrated that RIG-I contains ToH1 in close proximity to its nucleic acid binding site. This led us to hypothesize that HMGB1 binding to ToH1 in RIG-I supports detection of foreign nucleic acids during viral infection. Using biolayer interferometry, we detected direct interaction between RIG-I and HMGB1 that was not dependent on nucleic acids. We plan to further investigate this finding in cell-free and cellular studies of RIG-I activation in the presence or absence of HMGB1. Ultimately, findings from this study will provide critical information needed to understand the role of HMGB1 in viral infection and suggest new therapeutic strategies in viral diseases.

Abstract 27

Functional Comparison of Bat STING Orthologs to Understand Innate Immune Modulation in Reservoir Species

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Bats are reservoirs to many highly pathogenic viruses. Understanding how bat immunology facilitates asymptomatic infections is crucial for mitigating zoonotic diseases. STING is an innate immune protein shown to activate IRF3 and NF-kB, form a hydrogen ion pore leading to induction of autophagy, and initiate cell death. Previous studies with limited numbers of species have suggested that bat STINGs (bSTINGs) exhibit muted transcriptional responses yet maintain normal autophagic activity, potentially contributing to lower inflammation and viral clearance. However, bats are extremely diverse, and it is necessary to extend studies to a more expansive representation of orthologs to understand bat immune tolerance. We examined the functionality of 22 bSTINGs from seven families using four assays: IRF3 and NF-κB activation, autophagosome formation, and cell death induction. Orthologs within the same family typically exhibited similar functionalities. Yinpterochiroptera orthologs displayed IRF3 activation comparable to humans, whereas Yangochiroptera orthologs, except for *E. fuscus*, showed attenuated IRF3 response. All tested orthologs exhibited dampened NF-κB and autophagy functionality, except *E. fuscus* and *P. discolor* respectively. Cell death activation was varied and species specific. We hypothesize that bSTINGs autophagy has been dampened to hinder the activation of cell death, due to a slight correlation between cell death and autophagy. Furthermore, blocking the hydrogen ion pore attenuates autophagy and lowers cell death. Overall, our results show that testing a single species from a family may provide that family's specific phenotype for innate immune proteins, whereas to understand bat immunology as a whole, requires testing of representatives from multiple families.

Abstract 28

TNFRSF13B influences host immune response to enterobacteria in the mucosa

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TNFRSF13B governs T cell-independent antibody responses, plasma cell differentiation, and properties of immunoglobulin (Ig) A, G, and M. *TNFRSF13B* is extraordinarily polymorphic, but specific genotypes have been associated with common variable immunodeficiency (CVID). CVID afflicts 1 in every 25,000-50,000 people, however, this phenotype is exceptionally rare as these same genotypes occur in ~2% of the general population. This raises the question of how *TNFRSF13B* influences immune responses and what is the evolutionary benefit the gene locus having strong positive selection? Our studies in mice demonstrate distinct *Tnfrsf13b* polymorphisms alter antibody production, such as the decrease of secretory (s)IgA, and determine resistance and immunity of the host to enterobacteria. The research indicates that *Tnfrsf13b* variants determine both concentration of sIgA and effector functions of IgG that in turn promote either resistance or enhanced immunity to *C. rodentium*. In one mechanism, sIgA enhances virulence by activating the virulence program of *C. rodentium,* thus decreasing sIgA blocks infection from occurring. In another mechanism, *Tnfrsf13b* variants enhance the ability of IgG to activate complement that in turn helps clear the infection. We hypothesize that *TNFRSF13B* genotype shapes antibody quality, the intestinal environment, and host-microbe interactions, collectively influencing mucosal immunity in spite of decreasing sIgA. Together this research furthers our understanding of how *TNFRSF13B* regulates the kinetics of production and properties of IgA and how its genetic diversity contributes to host defense and immune homeostasis.

Abstract 29

Myosin 18A regulates B cell differentiation through restricting the unfolded protein response and TLR7-mediated signaling

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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 anergy and differentiate into antibody-secreting cells (ASCs). Actomyosin interactions are associated with B cell activation, yet no mechanisms connecting B cell cytoskeletal dynamics to B cell differentiation have been investigated. We previously generated a B cell-specific deletion of Myo18A (Myo18A BKO) and noted high concentrations of autoantibodies and a significant increase in *in vivo* and TLR7-induced *in vitro* ASCs, indicating that Myo18A restricts B cell differentiation, thus preventing secretion of autoantibodies. *In vitro* B cell differentiation does not differ in the timing of TLR7 stimulation or the proliferation generation in Myo18A-deficient B cells. Bulk RNA sequencing of mature, naïve Myo18A BKO and $Mb1^{Cre/+}$ B cells indicated a significant increase in genes associated with ER stress and the unfolded protein response (UPR), mechanisms necessary for B cell differentiation. An increase in ATF6 signaling was examined in naïve and TLR7-induced Myo18A BKO B cells. 48 hours post-TLR7 stimulation displayed transcriptome changes correlated with increased TLR7 signaling, indicating that greater differentiation of Myo18A-deficient B cells may be due to amplified TLR7 signaling. These findings indicate Myo18A restricts B cell differentiation through mediating the balance of ER stress and UPR and maintaining appropriate TLR7 signaling strength.

Abstract 30

MHC-II trafficking is impaired in Mtb-infected M2-like macrophages that evade CD4+ T-cell recognition

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), specializes in evading the immune response and requires efficient CD4+ T-cell activity for infection control. Recent evidence in mice shows that recognition of infected macrophages by Mtb-specific CD4+ T-cells is imperative for protection. We recently published that M1 but *not M2-like* macrophages efficiently activate Mtb-specific memory CD4+ T-cells upon infection with Mtb, whereas both successfully activate T cells when loaded with exogenous antigens or γ-irradiated Mtb. However, the mechanisms of subversion of T-cell response to infection in primary human macrophages remain unknown. In this study, we performed RNA sequencing on human M1 and M2-like macrophages exposed to either virulent Mtb or γ-irradiated Mtb. Pathway analysis of genes preferentially expressed by Mtb-infected M2-like macrophages showed enrichment of IL-10 signaling and Type I Interferon related genes such as *HERC5*. Mtb-infected human lung macrophages also showed enrichment of the Type I Interferon pathway and *HERC5*, and high levels of secreted IL-10. Infected M2-like macrophages showed impaired trafficking of intracellular MHC II to the surface and inhibiting either IL-10 signaling or knockdown of *HERC5* increased levels of surface MHC II post Mtb infection. Importantly, both IL-10 blockade and *HERC5* knockdown augmented activation of Mtb-specific T cell lines over Mtb-infected M2-like macrophages treated with either non-targeting siRNA controls and MHC II blockade.

Abstract 31

TGFBRAP1 Overexpression Selectively Inhibits Filovirus and SARS-CoV-2 Entry

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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 are CORVET specific subunits that mediate early endosomal fusion through associations with Rab5 GTPases. Conversely, the HOPS specific subunits, VPS39 and VPS41, induce late endosomal-lysosomal fusion. Preliminary data from a gene activating transposon mutagenesis screen for Ebola virus entry restriction suggested that TGFBRAP1 overexpression is antiviral. It is known the HOPS complex is utilized in filovirus entry because filoviruses 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 or VPS8 are overexpressed in HEK293T cells, HOPS complex formation is inhibited. The impact of the proportions of the 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. This phenotype is TGFBRAP1 overexpression dependent because siRNA knockdowns of TGFBRAP1 increased filovirus infectivity. In the context of TGFBRAP1 overexpression, knockdowns of VPS8, VPS39, or VPS41 didn't increase filovirus infectivity, suggesting that this phenotype is independent of the canonical CORVET complex. To further understand the impact of TGFBRAP1 overexpression in infections, we're testing if the entry protein cleavage events are impacted and where viral particles are being sequestered in these conditions.

Abstract 32

Inhibition of Type I Interferon Signaling Attenuates Acute Antibody-Mediated Rejection Without Altering Early Chronic Alloimmune Injury

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Background: Antibody-mediated rejection (ABMR) is a cause of kidney allograft failure. Our prior work showed that cathepsin G deficiency permits delayed emergence of donor-specific antibody (DSA)-associated autoantibodies contributing to chronic allograft injury, and NanoString nCounter analysis revealed reduced interferon-associated gene expression in allografts from CCR5$^{-/-}$ cathepsin G$^{-/-}$ recipients. While type I interferons (IFN-I) bridge innate and adaptive immunity, its role in acute and chronic ABMR remains unclear. We tested whether IFNAR (IFN alpha/beta receptor subunit 1) blockade—via anti-IFNAR antibody or IFNAR-knockout (KO) grafts—would attenuate ABMR and improve allograft survival.

Methods: Wild-type A/J or A/J.IFNAR$^{-/-}$ kidneys were transplanted into B6.CCR5$^{-/-}$ mice. A/J allograft recipients were treated with anti-IFNAR mAb or control rat IgG i.p. on post-transplant days 9, 12, and 15, beginning after serum DSA became detectable.

Results: Both IFNAR-KO allografts and anti-IFNAR mAb-treated recipients showed prolonged allograft survival versus controls (median survival: 50 and 50 vs. 29 days). DSA titers increased similarly across all groups with no significant difference in titers. At day 50, C4d staining revealed minimal deposition in anti-IFNAR-treated grafts and mild deposition in IFNAR-KO grafts, while IgG controls showed C4d positivity. CCR5$^{-/-}$ recipients of IFNAR-KO allografts exhibited markedly elevated autoantibodies against multiple autoantigens and collagen III/IV by ELISA from day 15 post-transplant compared with naïve controls. Histopathology at day 50 revealed mild fibrosis in both IFN-I signaling–targeted groups, indicating early chronic ABMR-associated injury.

Conclusions: IFN-I signaling critically drives acute ABMR. IFNAR blockade prevents acute ABMR but does not prevent progression to chronic ABMR.

Abstract 33

Condensin Dysregulation Drives Retrotransposable Element Derepression, Senescence, and Intestinal Barrier Loss

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Intestinal permeability is a hallmark of inflammatory bowel disease, including Crohn's disease, but the mechanisms that destabilize epithelial barrier homeostasis remain poorly understood. Because barrier integrity depends on intestinal stem cells that sustain epithelial turnover and repair, we asked whether elevated condensin expression disrupts gut homeostasis. We found that the condensin II subunit NCAPD3 is overexpressed in ileal epithelial stem cell populations from Crohn's disease patients.

To test the functional consequences of this in vivo, we overexpressed the NCAPD3 homolog dCAPD3 in *Drosophila melanogaster* midgut stem cells and observed increased intestinal permeability in 10-day-old adults. dCAPD3 overexpression also induced heterochromatin reorganization, stem cell senescence, apoptosis, and inflammation, including SASP-like signaling in stem cells and increased immune gene expression, with damage extending to neighboring non-stem cells, consistent with both cell-autonomous and non-cell-autonomous effects.

Previous work showed that condensins are required for stem cell integrity in the Drosophila brain and repress retrotransposable elements (RTEs) in somatic tissues. We therefore asked whether altered condensin dosage in gut stem cells similarly disrupts RTEs control and barrier homeostasis. Interestingly, we found that dCAPD3 overexpression increased RTE expression as detected by qPCR and RNA FISH. Because RTEs activation is known to promote cellular senescence, these findings suggest that RTEs derepression may contribute to the senescent phenotype observed in dCAPD3-overexpressing stem cells. Blocking RTEs activity with AZT partially rescued barrier dysfunction, and overexpression of mdg4 also increased gut permeability. Together, these findings support a model in which condensin dysregulation drives RTEs derepression, senescence-associated inflammatory signaling, stem cell dysfunction, and intestinal barrier loss.

Abstract 34

AI-enhanced workflow for quantification of cell death in the mouse small intestine

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The mammalian small intestine is a feature-rich barrier tissue that interfaces with the external environment to balance nutrient uptake and pathogen defense. Its architecture includes densely folded villi that maximize absorption in the intestinal lumen and crypts that house proliferating stem cells. Quantifying histological features such as increased cell death can provide insights into tissue health and disease pathogenesis. Apoptosis, a form of programmed cell death, is histologically characterized by cell contraction, hyper-eosinophilia, and nuclear condensation (pyknosis) and fragmentation (karyorrhexis). Despite these notable features, apoptotic bodies can be challenging to distinguish from mitotic cells or intraepithelial lymphocytes (IELs). Our goal is to move beyond manual annotation, which is prone to observer bias and is labor-intensive. We implemented an artificial intelligence (AI)-enhanced image analysis pipeline using *EZannot*, a Python-based tool for annotation and segmentation. We trained an Annotator within *EZannot* to identify apoptotic bodies in H&E-stained intestinal tissue of mice with excess crypt apoptosis induced by EdU. We compared accuracy and time between the AI-assisted pipeline, a novice annotator, and an experienced histologist (gold standard). Notably, AI-quantification of apoptotic bodies in ~1 cm of small intestinal tissue (n = 24 mice) showed high concordance with manual quantification (r = 0.96). These results suggest that AI-based tools can be used to quantify apoptotic bodies in the mouse small intestine. By reducing annotation time and minimizing subjectivity, this pipeline shows promise in quantifying additional histological features that may accelerate the understanding of intestinal injury in mice and pathology of human gastrointestinal diseases.

Abstract 35

Monoclonal Antibody 5C12 is an Allosteric Inhibitor of Factor XIIa's Active Site

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Background: Engineered blood-contacting medical devices are essential for patient management. However, medical device-associated thrombosis is common. Inhibitors to FXIIa have been proposed as an alternative to heparin to prevent medical device-associated thrombosis without increasing the risk of bleeding. The monoclonal antibody (Mab), 5C12, is a potent anti-FXIIa antibody in development to prevent medical device-associated thrombosis. We mapped the epitope of 5C12 on α-FXIIa.

Results: 5C12 is a conformationally specific Mab. It immunoblots FXII and FXIIa only on non-reduced samples. 5C12 preferentially binds to α-FXIIa over FXII. α-FXIIa blocks 5C12 binding to α-FXIIa linked to microtiter plates with an IC_{50} of 11.78 nM and it inhibits α-FXIIa hydrolysis of H-D-Pro-Phe-Arg-pNA·2HCl with an IC_{50} of 3.5 nM. Through a series of iterative peptide preparations using the crystal structure of FXIIa as a guide, we determined that 5C12 binds to a 5 amino acid external loop I^{383}APCW on FXIIa that is near the active-site H^{393}. The $C^{386}W^{387}$ sequence is highly conserved in the FXII family of proteins: tPA, uPA, and HGFA. Competition inhibition assays using peptides IAPAW and IAPCA reveal that the cysteine and tryptophan in α-FXIIa are critical for 5C12 binding. Recombinant FXII (rFXII) is activated by plasma kallikrein and competes Mab 5C12 binding, whereas $rFXII_{IAPCA}$ and $rFXII_{IAACW}$ have low chromogenic activity and cannot compete Mab 5C12.

Conclusion: Mab 5C12 binding to FXIIa changes the orientation of the active site histidine, incapacitating access to the catalytic triad. Using anti-FXIIa Mabs instead of heparin have potential to prevent thrombosis on medical devices.

Abstract 36

Immune-Metabolic Crosstalk: NO-Driven Regulation of Cytochrome P450

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The functional maturation of cytochromes P450 (CYP) 3A4 and 2D6, enzymes responsible for metabolizing over 50% of clinical drugs, is a critical yet poorly understood process. While immune activation is known to alter CYP catalytic activities, the precise mechanisms governing cellular heme delivery and insertion into these enzymes remain largely undefined. We investigated the impact of nitric oxide (NO), a key effector molecule in the innate immune response on CYP heme allocation using mammalian cell lines transiently transfected with CYP3A4 or CYP2D6. NO exposure was achieved via chemical donors and endogenous production from immune-stimulated macrophages. Our research reveals that NO operates via a bimodal regulatory mechanism. Specific low concentrations of NO (approximately 1-10nM), such as those produced by activated macrophages stimulate the GAPDH–heme complex to increase active CYP levels by 2-3 folds. Conversely, higher concentrations (approximately 25-100nM) typical of chronic inflammatory diseases like sepsis or rheumatoid arthritis, trigger heme loss and a rapid decline in enzyme activity, creating a direct link between the immune microenvironment and systemic drug metabolism. This regulation occurs without affecting CYP protein expression. Mechanistically, this NO-driven heme transfer involves delivery from a GAPDH-heme complex and requires the functional activity of the cellular chaperone Hsp90. These findings identify a novel immunometabolic checkpoint where NO dynamically tunes CYP activities by modulating heme flux which in turn alters drug metabolism and the production of immune-active metabolites like prostaglandins, offering critical insights into the intersection of inflammatory signaling and systemic metabolic function.

Abstract 37

Macrophage Piezo1 Drives Myofibroblast Differentiation through Cell-Cell Contact

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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. In the setting of emerging data implicating immune processes in fibrosis, we sought to determine if Piezo1 mediates pro-fibrotic macrophage-fibroblast crosstalk. Pro-fibrotic macrophage-fibroblast crosstalk has been shown to be dependent on both soluble factors and cell contact dependent mechanisms. We first tested the role of Piezo1 on secretion of a soluble factor to drive myofibroblast differentiation. To test this, upon transfer of c**onditioned media (CM) from** Piezo1$^{fl/fl}$ **or** Piezo1^{LysMcre} bone **ma**rrow-derived macrophages (BMDMs) to WT mouse lung fibroblasts (MLFs), myofibroblast differentiation was measured. Piezo1$^{fl/fl}$ **and** Piezo1^{LysMcre} BMDMs induced myofibroblast differentiation to a similar degree. Next, to determine the role of macrophage Piezo1 on cell contact dependent myofibroblast differentiation, we plated BMDMs and MLFs in co-culture. Co-culture with Piezo1$^{fl/fl}$ increased myofibroblast differentiation similar to TGF-β treated controls, while Piezo1^{LysMcre} BMDMs failed to induce myofibroblast differentiation. Taken together, this data suggest that macrophage Piezo1 drives myofibroblast differentiation through cell-cell contact. To determine the macrophage-fibroblast contact dependent mechanism, bulk RNA sequencing revealed increased expression of the anti-fibrotic gene Syndecan-2 (Sdc-2) in Piezo1 depleted macrophages. Furthermore, our *in vivo* preliminary data show, utilizing a non-resolving bleomycin mouse model of IPF, myeloid-specific Piezo1 depletion protects the mouse from pulmonary fibrosis. Mechanisms by which macrophage Piezo1 drives macrophage-fibroblast crosstalk are a necessary prerequisite to identify novel targetable pathways to ameliorate fibrosis in IPF.

Abstract 38

Understanding How Human STING Polymorphisms Modulate SAVI-Variant Functionality

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Inborn errors of immunity are mutations to key proteins involved in immune system function, often leading to immune-mediated pathogenesis. One such example involves dysregulation of the innate immune protein STING. Gain-of-function mutations in STING cause the autoinflammatory disease known as STING-Associated Vasculopathy with onset in Infancy (SAVI). SAVI mutations cause constitutive activation of STING and its downstream pathways, resulting in chronic inflammation, interstitial lung disease (ILD), and fevers, often leading to death. Current treatments that target interferon signaling are limited in availability and effectiveness against ILD, the leading cause of death in SAVI, suggesting that other STING functions are involved in disease progression. Additionally, little is known about how the background of common STING alleles modulate SAVI-variant functionality and disease progression. In this study, we characterized the functionality of four known SAVI variants in the backgrounds of the wild-type R232 and HAQ alleles, specifically looking at IRF3 and NF-κB transcriptional activity, as well as autophagosome formation. We found that the HAQ allele protected against basal activation of IRF3 and NF-κB activity relative to the R232 allele, and also suppressed basal autophagy induction. Additionally, we found that the HAQ allele inhibited activation of two of the four SAVI variants across these pathways even in the presence of stimulus. By systematically characterizing these variants across all of the common STING alleles, we aim to better understand how genetic backgrounds influence disease progression, and provide insight to inform clinicians in providing more effective therapeutics to SAVI patients.

Abstract 39

Nasal Mucosal Recall Dominance by Recirculating Memory CD8$^+$ T Cells

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Respiratory viruses such as influenza and SARS-CoV-2 typically transmit within the upper airway, placing the nasal mucosa at the frontline of antiviral defense. CD8$^+$ T cells provide cross-strain protection by recognizing conserved viral epitopes and promoting rapid viral clearance, making them attractive targets for next-generation vaccines. Recent strategies have focused on inducing nasal tissue resident memory CD8$^+$ T cells (CD8$^+$ T_{RM}), which persist locally and block viral dissemination. However, the nasal cavity is also a specialized sensory organ, and immune activity at this site may compromise olfactory function as highlighted by the widespread smell loss observed during the COVID-19 pandemic. Defining how antiviral immune responses intersect with sensory biology is therefore critical for developing interventions that protect against infection without impairing sensory health. We developed an experimental platform allowing the targeted depletion of nasal CD8$^+$ T_{RM}. Leveraging this tool, our preliminary data reveal that recirculating memory CD8$^+$ T cells, rather than nasal CD8$^+$ T_{RM}, dominate antigen-specific recall responses in the nasal mucosa and can independently drive olfactory dysfunction. These findings challenge the prevailing view that nasal CD8$^+$ T_{RM} are critical mediators of upper airway recall immunity and reveal an unexpected role for systemic memory in regulating olfactory function.

Abstract 40

Human memory CD4$^+$ T cells recognize non-infected macrophages bystander to *Mycobacterium tuberculosis* infection

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Direct recognition of *Mycobacterium tuberculosis* (Mtb)-infected macrophages by CD4+ T cells is required for the control of infection. Within granulomas, T cells consistently co-localize with non-infected macrophages, raising the possibility that these bystander cells also present Mtb antigens to T cells. To test the hypothesis, we developed an ex vivo co-culture system pairing memory CD4+ T cells from healthy individuals with latent Mtb infection (LTBI) with autologous, non-infected monocyte-derived macrophages (MDMs), using flow sorting and transwell plates to physically separate bystander from infected macrophages. Memory CD4$^+$ T cells co-expressed activation-induced markers (AIMs) in response to bystander macrophages in a TCR-pMHC-II dependent manner, but with reduced IFNγ and IL-2 expression compared to responses to infected macrophages. TCR sequencing identified Mtb-specific clonotypes among bystander-activated CD4$^+$ T cells, and antigen-specific recognition of bystander macrophages was confirmed using TCR-transduced cell lines. Bystander macrophage activation of T cells persisted despite removal of extracellular vesicles (EVs) from conditioned supernatants where multiple secreted Mtb antigens were detected by mass spectrometry, implicating soluble antigen transfer as the likely mechanism. Together, these findings demonstrate that non-infected bystander macrophages elicit antigen-specific human CD4$^+$ T cell responses, albeit with a diminished effector cytokine profile. We post that soluble Mtb antigen transfer to bystander macrophages decoys T cells away from infected cells, potentially undermining protective CD4 responses.

Abstract 41

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

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Obesity is a prevalent chronic disease, affecting more than 2 in 5 U.S. adults, and its impact on immune function has become a critical concern. Evidence indicates that individuals with metabolic diseases, including obesity, exhibit impaired humoral immune responses, with vaccine-induced immunity waning more rapidly in this population. Effective humoral immunity depends on the generation of short- and long-lived plasma cells (PCs) as well as memory B cells, processes that are tightly regulated by B cell-intrinsic metabolism. We hypothesize that elevated basal metabolic activity in naïve B cells in the context of obesity drives a bias toward rapid PC differentiation at the expense of germinal center reactions, resulting in fewer high-affinity long-lived PCs and memory B cells. Preliminary data support this model: wild-type mice fed a high-fat diet generated fewer long-lived, high-affinity PCs following immunization, and gene expression analysis revealed that PCs from these animals displayed a more immature phenotype. Ongoing studies aim to identify differentially regulated metabolic pathways and assess the cell-intrinsic effects of diet-induced obesity on B cell responses across diverse vaccine platforms. We further hypothesize that by modulating adjuvant strength and antigen persistence, it may be possible to fine-tune these responses to enhance humoral immunity in populations affected by obesity.

Abstract 42

Recipient Monocytes Are Required for Heterologous Donor-Reactive Memory CD8 T Cell Activation to Reject High Risk Allografts

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We have demonstrated that extending allograft cold ischemic storage (CIS) markedly increases early inflammation, leading to activation of endogenous donor-reactive CD4 and CD8 T cells as well as neutrophils, macrophages and Ly6C+ monocyte in heart allografts and promotes CTLA-4Ig resistant rejection of the higher risk allografts. Whether and what recipient derived myeloid cells play a critical role in early inflammatory responses have not been fully elucidated. To investigate the role of recipient monocytes in endogenous memory T cell activation in allografts, we transplanted complete MHC mismatched A/J allografts subjected to 8hrs CIS into chimeric B6.CD11b diphtheria toxin receptor (DTR) transgenic recipients and administered DT to deplete bone marrow derived CD11b+ cells prior to the transplant. Depletion of recipient CD11b+ cells inhibited the infiltration and proliferation of early graft infiltrating endogenous memory CD4 and CD8 T cell on day 2 post-transplant and markedly extended survival of highly ischemic allografts in CTLA-4Ig conditioned recipients. Prolonged CIS also induced increases in allograft IL-1β and TNFα mRNA expression that were decreased in high-risk allografts from CD11b+ cell depleted recipients. While expression of TLR9 but not TLR4 or TLR7 mRNA was increased in high-risk allografts on day 2 post-transplant, its expression was decreased in the absence of recipient CD11b+ cells. Collectively, these results indicate graft-infiltrating recipient inflammatory monocytes in sustaining the early post-transplant inflammation to activate endogenous donor-reactive memory CD8 T cells to mediate acute graft injury and CTLA-4Ig resistant rejection.

Abstract 43

Intestinal epithelial cell (IEC)-derived gasdermin C (GSDMC) regulates IL-33 subcellular trafficking during chronic intestinal inflammation

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Inflammatory bowel disease (IBD) is a chronic, multifactorial disorder of the gastrointestinal tract in which IEC dysregulation is implicated in disease pathogenesis; however, their mechanistic contributions remain unknown. Increasing evidence indicates the importance of gasdermin (GSDM) proteins in the pathophysiology of GI-related diseases, including IBD, specifically for their function(s) in IECs. GSDMC is highly expressed in IECs and upregulated in a context-dependent manner; however, its contribution to chronic intestinal inflammation has also not been investigated. Our preliminary data show inherent and robust upregulation of *Gsdmc2-4* prior to the onset of inflammation (at 4 wks) in ileitis-prone SAMP1/YitFc (SAMP) mice compared to age-matched AKR controls, which further increases when disease is established (at 20 wks). Additionally, we observe an IEC-subtype specific alteration in *Gsdmc2-4*, particularly in enterocytes and intestinal stem cells (ISCs) vs. AKR. Not only is GSDMC expression altered as disease increases, but so is its activation. IF 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 AKRs. Notably, GSDMC accumulates at the nuclear membrane, and IL-33 in the nucleus, of IECs. Likewise, subcellular fractionation from 20-wk old SAMP indicates increased expression and nuclear localization of full length and cleaved GSDMC compared to 4-wk SAMP and age-matched AKR controls. Taken together, our results suggest that GSDMC plays a critical regulatory role in the bi-directional translocation of IL-33 into/out of the nucleus during chronic intestinal inflammation, such as that observed in IBD.

Abstract 44

Estrogen Receptor–Dependent Regulation of IgG Glycosylation

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Immunoglobulin G (IgG) Fc-domain glycosylation critically regulates antibody effector function, with decreased α2,6-linked sialylation consistently associated with autoimmune disease and inflammation. In contrast, IgG sialylation increases during pregnancy and is associated with remission of autoimmunity and inflammation, yet these are reversed soon after parturition. We have previously shown that endothelial cells (ECs) act as systemic regulators of IgG sialylation by internalizing circulating IgG and remodeling Fc glycans during neonatal Fc receptor (FcRn)-mediated recycling. However, the pregnancy-associated signals and their impact on EC-mediated IgG sialylation remain poorly understood. Estrogen (E2) is a strong candidate regulator of this process, as its levels increase dramatically during pregnancy and previous reports suggest a connection between E2 levels and IgG sialylation even outside of pregnancy. It is also known that signaling through nuclear estrogen receptors alpha (ERα) and beta (ERβ) mediates transcriptional programs that can shape immune tone. Consistent with this model, our preliminary data indicates that deletion of either ERα or ERβ leads to reduced IgG sialylation. *In vitro* studies using human ECs (HMEC-1) demonstrate that ERα- and ERβ-selective agonists differentially regulate expression of the sialyltransferase ST6Gal1 compared to E2, further suggesting a role for E2 in regulating IgG sialylation. We therefore hypothesize that ERα and ERβ play distinct but poorly understood roles in regulating IgG sialylation by controlling sialyltransferase expression in the endothelium lining the vasculature. Our ongoing work is focused on defining the mechanistic link between endocrine signaling, antibody sialylation and immune quiescence.

Abstract 45

Microbiota-derived metabolite loss drives Th17-type immune responses in a murine model of Crohn's disease (CD)-like ileitis

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Inflammatory bowel disease (IBD), including ulcerative colitis and CD, is a chronic immune-mediated disorder arising in genetically susceptible individuals, influenced by environmental factors including the gut microbiome. Although microbial dysbiosis is implicated in disease pathogenesis, the mechanisms linking it to pathogenic immune responses remain unclear. This study investigates how the microbiome drives immune dysregulation in ileitis-prone SAMP1/YitFc (SAMP) mice, a spontaneous model of CD-like ileitis. Preliminary 16S sequencing of fecal samples from SAMP and AKR/J (parental control) mice shows reduced α-diversity in SAMP, while β-diversity reveals distinct clustering between strains, indicating divergent microbial communities. Relative abundance analysis shows reduced members of *Clostridia* and *Bacteroidetes* phyla, producers of short-chain fatty acids (SCFAs) and bile acids (BAs), demonstrating loss of commensals in SAMP. Untargeted bulk tissue metabolomics on ilea and fecal samples comparing SAMP vs. AKR/J reveal distinct metabolomic profiles, with concordant depletion of SCFAs, BAs, and BA conjugates. Immunophenotyping of unfractionated mesenteric lymph node cells shows a Th17-dominant cytokine profile in SAMP, with increased IL-17A and IL-22. Investigating the cellular source of these Th17-type cytokines reveals an increased ratio of Th17 cells and IL-17-producing regulatory T cells (Tregs) relative to conventional Tregs. While no differences in absolute numbers of IL-17-producing $\alpha\beta^+$ T cells are detected, IL-17-producing $\gamma\delta^+$ T cells trend higher in SAMP. Collectively, these findings link loss of *Clostridium* and *Bacteroidetes* to reduced SCFAs and BAs, promoting a pro-inflammatory profile. As these metabolites support Treg stability and constraint Th17 differentiation, their loss is consistent with a shift towards Th17-dominant immunity.

Abstract 46

Co-operation between gasdermin (GSDM) family members, GSDMB and GSDMD, may regulate goblet cell function during homeostasis and is dysregulated during inflammatory bowel disease (IBD)

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GSDMs are a family of structurally-related proteins mainly known for their role in pyroptosis, and whose dysregulation has been reported in IBD. Our group reported GSDMB upregulation in intestinal epithelial cells (IECs), specifically colonocytes/crypt top colonocytes, of ulcerative colitis (UC) patients vs. healthy controls; however, a significant increase in goblet cells (GCs) was also observed, but its function(s) in GCs has not yet been explored. GCs are specialized IECs that produce and secrete mucins and antimicrobial peptides (AMPs) that are critical to maintain gut homeostasis. The aim of this study is to determine GSDMB-dependent function in GCs during healthy vs. disease states (*i.e.*, chronic intestinal inflammation). By scRNA-Seq, *GSDMB*/*GSDMD* are increased in GCs from UC inflamed vs. non-inflamed colonic mucosa, while their co-localization and upregulation is observed in UC-derived organoids. Notably, GSDMB-dependent GSDMD regulation is seen in WT vs. *GSDMB*$^{-/-}$ IECs, and preliminary data indicate differential expression of isoform-specific *GSDMB* in HT29+MTX (mainly GSDMB-416 and -407) vs. LS174T (mainly GSDMB-403) GCs. In fact, genes related to defense molecules, including *LYZ*, are differentially regulated in WT vs. *GSDMB*$^{-/-}$ LS174T cells, while *REG4* is differentially regulated in WT vs. *GSDMB*$^{-/-}$ HT29 cells. Interestingly, mice overexpressing GSDMB (*i.e.*, GSDMB-411$^{IEC\text{-}Tg}$) vs. WT controls produce and release copious amounts of mucus, with robust accumulation, which is recapitulated in *ex vivo* organoids. These data suggest GSDMB/GSDMD upregulation and co-operation in GCs, with potential functional relevance of GSDMB and its isoform(s), specifically regarding defense molecule synthesis/secretion during chronic intestinal inflammation, such as in IBD.

Abstract 47

Identifying Immune Correlates of Protection to *Plasmodium vivax* using Human Monoclonal Antibodies to Apical Membrane Antigen 1

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One-third of the human population, mainly in South America and Southeast Asia, is at risk of contracting *Plasmodium vivax* (Pv) malaria. Apical Membrane Antigen 1 (AMA1) is a critical surface protein that binds to Rhoptry Neck Protein 2 (RON2); this interaction is utilized by both sporozoites and merozoites and is vital for invading liver cells and reticulocytes, respectively. PvAMA1 is a highly immunogenic surface antigen, and individuals with high antibody (Ab) levels to AMA1 are associated with a decreased risk of malaria. We have identified two PvAMA1-specific human monoclonal antibodies (humAbs), 826827 and 864865, that target epitopes on Domain 1 of AMA1 and inhibit Pv infection *both in vitro* and *in vivo,* with cross-strain blocking effects. Competitive ELISA using these humAbs shows that a certain percentage (6 to 73%) of individuals from various Pv-endemic areas develop blocking antibodies, ranging from 33.3 to 97% blocking of 826827 and from 36 to 99% blocking of 864865. Children under 12 demonstrate more blocking of 826827 compared to 864865, while adults exhibit greater blocking of 864865. We hypothesize that individuals with the highest levels of blocking antibodies against these epitopes have a lower risk of Pv infection and disease. These analyses are ongoing through our well-established longitudinal cohort studies of Pv in Papua New Guinea, the Solomon Islands, and Brazil. These findings will support the use of these humAbs for preventative therapy to stop Pv infection and aid in developing a Pv vaccine that includes these epitopes, thus targeting both pre-erythrocytic and blood-stage infections.

Abstract 48

C9orf72 controls the balance of Th17 and T regulatory cell fate choice

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There is substantial evidence that the balance of pro-inflammatory T helper type 17 (Th17) cells and immunosuppressive T regulatory (Treg) cells plays an important functional role in the pathophysiology of FTD and the related motor neuron disease Amyotrophic lateral sclerosis (ALS). FTD/ALS patients with a rapid disease course have elevated ratio of Th17:Treg cells in circulation and the inflammatory cytokine Interleukin 17A (IL-17A) produced by Th17 cells is elevated in patient blood and cerebral spinal fluid (CSF). Infusion of autologous Tregs have slowed motor decline in ALS patients in clinical trials; however, the infused Tregs did not persist long-term, and beneficial effects lasted only a few weeks. This suggests that environmental factors in patients contribute to Treg instability and/or functional re-programming. Here we provide evidence that C9orf72 acts within naive T cells to control the decision to become Treg or Th17. An improved understanding of genetic and environmental modifiers of T cell fate and function in FTD/ALS patients has potential to improve current and future T cell-directed therapies in the clinic.

Abstract 49

Perturbed neutrophil responses in *Irf3*-deficient mice protect from CCl_4-induced liver fibrosis in mice

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Interferon regulator factor 3 (IRF3) executes multiple transcriptional and non-transcriptional functions in the development of metabolic liver diseases. While *Irf3*-deficient mice are protected from liver fibrosis, the relative contributions of IRF3 activities are unknown. Liver fibrosis is characterized by excessive extracellular matrix accumulation and involves recruitment and activation of innate immune cells. Neutrophils play a dual role in fibrosis, promoting fibrosis through cytokines and granule proteins while also degrading matrix. Yet neutrophils regulation by IRF3 is largely unstudied in liver. Therefore, we aim to further investigate the role of neutrophils in liver fibrosis and assess the contribution of IRF3 to neutrophil responses.

C57BL/6J (WT), *Irf3*-deficient ($Irf3^{-/-}$), and mice expressing only non-transcriptional IRF3 function ($Irf3^{S1/S1}$) were exposed to CCl_4-induced liver fibrosis. Mice were treated with anti-Ly6G antibody to deplete neutrophils. Primary neutrophils were isolated from control mice and stimulated with PMA to induce degranulation and NETosis.

Chronic CCl_4 triggered robust infiltration of neutrophils into the liver. Interestingly, neutrophil accumulation was higher in $Irf3^{-/-}$ mice compared to both WT and $Irf3^{S1/S1}$ mice, but NET formation, assessed by citrullinated histone (CitH3) staining was impaired. Neutrophil depletion reduced the protective effect of *Irf3*-deficiency on CCl_4-induced fibrosis. Similar to the *in vivo* response, NET formation, evidenced by accumulation of extracellular DNA and CitH3 staining, and MPO release were reduced in neutrophils isolated from $Irf3^{-/-}$ compared to neutrophils from WT and $Irf3^{S1/S1}$. Together, these results suggest that the non-transcriptional function of IRF3 contributed to critical dependent neutrophil functions, likely resulting in progression of liver fibrosis.

Abstract 50

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

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B cells and the antibodies they produce are a major hurdle to the success of organ transplantation. In spite of immunosuppression all recipients develop donor-specific B cell responses (DSBs) that specifically recognize and respond to alloantigens expressed by the transplanted graft, including mismatched donor human leukocyte antigen (HLA) molecules. Even though DSB cell responses develop in every recipient only a few undergo antibody-dependent rejection. There are currently no tests that allow the determination of what DSB cell responses are likely pathogenic and which are not. We have found that common variants of *TNFRSF13B* a gene that encodes TACI are associated with antibody immunopathogenesis. We hypothesize that common TACI variants determine DSB cell evolution increasing affinity maturation by focusing intraclonal evolution. Objective: To develop and test a novel bioinformatics analysis tool to distinguish DSB clonal evolution associated with immunopathogenesis from more benign DSB clonal evolution in association with TACI variants.

PBMC samples from 10 recipients that received kidneys from living donors were analyzed. All 10 recipients were treated with standard immunosuppression. Six of the recipients received induction therapy and four did not. None developed donor-specific antibodies during the observation period, all were compliant with immunosuppression, and samples were collected longitudinally. 2 recipients expressed the P251L TACI variants and had cell free DNA and histologic evidence of graft damage post-transplantation. DS-B cells were isolated from PBMCs by panning on lightly fixed fibroblasts grown from tissue collected at transplantation. DSB were obtained before transplant and at 3,6 and 12 M thereafter. BCR sequences were processed using pRESTO from raw sequencing FASTQ files to generate high-quality reads. Assignment of V(D)J genes, CDR3 regions, trims of non-VDJ sequences, and assignment of clones based on shared V gene, J gene, and similar junction (CDR3) sequences was done by Change-O using IgBLAST results to annotate. Clonal assignment was refined by grouping sequences whose junction regions have a normalized Hamming distance below a defined threshold. For each clone within each sample, only clonotypes containing identical sequence-aligned BCRs observed in more than one instance were retained for downstream analysis. Pairwise Hamming distances were calculated between unique sequence alignments within each clone. A consensus germline sequence was reconstructed, and a neighbor-joining tree rooted on the germline was generated to represent clonal lineage relationships. Branches supported by only a single sequence differing by one Hamming distance were pruned to reduce noise as they likely reflect sequencing errors. For each clone, the Hamming distance between the consensus sequence and each mutated sequence was computed. The frequency distribution of these distances was summarized as a histogram to compare mutation accumulation patterns across samples before and after transplantation.

Results show that the distribution of the mutation frequencies in 3 of the 10 samples either did not change significantly before and after transplantation or clones became narrower after transplantation. Two of the 3 samples had a P251L mutation on TNFRSF13B. In contrast the distribution of the mutation frequencies widened after transplantation (relative to before transplantation) in the 7 recipients that maintained healthy graft function throughout the post transplantation period. Our findings suggest that DSB cell intra-clonal evolution is associated with graft health and that variants of TNFRSF13B determine DSB cell intra-clonal evolution and in this way the likelihood of antibody-mediated graft disease.

Abstract 51

Conformation analysis of STAT1 homodimers reveal the immuno-incompetence mechanism in STAT1 GOF patients

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Heterozygous gain-of-function (GOF) mutations in signal transducer and activator of transcription 1 (STAT1) cause a severe inborn error of immunity characterized by a combination of recurrent infections and autoimmunity. Current molecular hallmark is elevated phosphorylated STAT1 following interferon stimulation. While over 120 mutations have been identified, the genotype-phenotype correlation remains unclear due to high clinical heterogeneity. Canonically, tyrosine-phosphorylated STAT1 undergoes a conformational rearrangement from antiparallel to parallel homodimer configuration, initiating transcription. Antiparallel dimers are believed to negatively regulate STAT1 transcription by facilitating tyrosine dephosphorylation. Notably, most STAT1 GOF mutations localize to the interface of antiparallel dimers, suggesting these mutations may disrupt STAT1 conformational dynamics. Here, we developed the first live-cell, real-time NanoBiT assay to monitor STAT1 conformational dynamics in response to cytokines and clinically used inhibitors. Our results demonstrated these mutations exhibited diverse characteristics, with varying degrees of disrupted antiparallel dimers, phosphorylation levels, and conformational dynamics. We then categorized them and most mutations clustered in one group, displaying hyperphosphorylation, and reduced antiparallel dimers. However, the remaining mutations displayed distinct or even opposite features. Transcriptional analysis showed certain mutations with high phosphorylation exhibited reduced expression of interferon-stimulated genes. Moreover, several mutations exhibited resistance to inhibitor-mediated dephosphorylation. Altogether, our findings suggest that STAT1 GOF pathogenesis cannot be fully explained by elevated STAT1 phosphorylation, but is more likely driven by disrupted STAT1 conformational dynamics. Our NanoBiT-based system provides a powerful tool to dissect the molecular pathogenesis of the disease, and has the potential to develop personalized treatment options by restoring balanced STAT1 dynamics.

Abstract 52

EphA2 receptor tyrosine kinase mediates pan-cancer immune evasion

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EphA2 is overexpressed in human solid cancer, which is correlated with poor prognosis. However, the underlying mechanisms remain incompletely understood. We find EphA2 knockout led to reduced tumorigenesis across multiple syngeneic mouse models including lung, skin, colon, liver and breast cancer. Immune profiling, RNAseq, cytokine analysis and spatial omics show the recruitment and activation of CD8 T cells and upregulation of MHC-I, which is linked to CCL5 and CXCL9/10 upregulation and IFNγ signaling. EphA2 binds to membrane-tethered ligands including ephrin-As, and mediate cell-cell contact signaling. Interestingly, codeletion Efna1;Efna3;Efna4 genes that encode ephrin-A1, -A3 and A4 ligands for EphA2 led to markedly reduced tumorigenesis associated with increased antigen processing and presentation. Our studies uncovered a hitherto unrecognized novel immune evasion mechanism mediated by the interaction between ephrin-A ligand on host immune cells and EphA2 on tumor cells. Finally, induced degradation of EphA2 suppressed tumor development and synergized with anti-PD-L1 treatment, demonstrating potential clinical utility of our discoveries.