Case Western Reserve University School of Medicine

Department of Pathology

17th Annual Immunology Retreat

and

The Enrique Ecker Memorial Lecture

Friday, April 26, 2024
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 17th 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. The current iteration of this retreat represents the completion of rebranding from a PhD program-specific retreat into a local immunology retreat, a goal I have been working towards for several years. The hope is to bring together all local investigators interested in immunology regardless of programmatic affiliation.

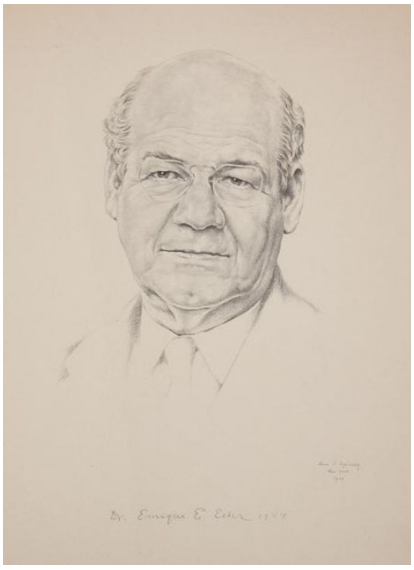
This year we have incorporated the Enrique Ecker Memorial Lecture into the program. Our Ecker Lecturer this year is Dr. Avery August, Howard Hughes Medical Institute Professor at the Cornell University College of Veterinary Medicine. Dr. August is a world-renowned expert on the role of tyrosine kinases in regulating the immune system.

Finally, I would like to personally thank everyone on the planning committee: Drs. Wendy Goodman, Anna Bruchez, Brian Gaudette, Doug Brubaker, and Rob Fairchild. I'd also like to thank the administrative staff: Christy Kehoe, Sophie Roussel-Kochheiser, and Andrea Shellenberger for helping make the arrangements. I also need to thank all of the departments and divisions, especially Dr. Thad Stappenbeck for co-sponsoring this event. And of course, I need to thank our Department Chair and Cheerleader-in-Chief Dr. Cliff Harding, and all of our participants for making this event an outstanding way to spend a springtime Friday.

Thank you!

Brian A. Cobb, PhD
Director, Immunology Training Program

Enrique Ecker



Dr. Ecker started his research program at Case in the Institute of Pathology in 1921 and authored an early textbook entitled Principles of Immunology with the head of the Institute at the time – Howard Karsner. Dr. Ecker is known for his fundamental work on endotoxin and the complement cascade. He also worked on tetanus toxin and diphtheria toxin which led to the modern vaccine formulations.

In complement, his early work defined C4 and the order of the classical pathway. Together with his student Louis Pillemer, he later discovered the alternative complement pathway while another student of his, Irwin Lepow purified some of the components of the pathway to characterize the enzymatic activity of the complement cascade. Enrique Ecker was the first in a long series of leaders in immunology and specifically the complement field here at Case Western Reserve University.

This lecture was started in 1958 and includes many distinguished immunology investigators. Earlier lecturers include Drs. Frank Dixon, Max Cooper, Emil Unanue, Victor Nussenzweig, and Jeff Revetch, with other more recent lecturers listed below. The Ecker Lecture Award encourages and celebrates excellence in fundamental and applied immunology and serves as a permanent tribute to our former faculty colleague in the Department of Pathology at Case Western Reserve University.

Recent Ecker Lecturers

2009	Alexander Rudensky
2010	Michel Nussenzweig
2011	Diane Mathis
2012	Betty Diamond
2015	Ellen Rothenberg
2017	David Scadden
2018	Irving Weisman
2021	Gwendalyn Randolph
2022	James Anderson
2024	Avery August

2024 Ecker Memorial Lecture Speaker

Avery August, PhD

Howard Hughes Medical Institute Professor, and Professor of Immunology
Cornell University College of Veterinary Medicine
Ithaca, NY, USA

Dr. August is Professor and former Chair of the Department of Microbiology and Immunology. He is currently HHMI Professor and Deputy Provost. His previous position was as Distinguished Professor of Immunology in the Department of Veterinary & Biomedical Sciences, and Director of the Center for Molecular Immunology & Infectious Disease, at The Pennsylvania State University at University Park, where he started as an Assistant Professor.

He received a BS degree in Medical Technology from the California State University at Los Angeles, and a PhD degree in Immunology from the Weill Cornell Graduate School of Medical Sciences. He was a Postdoctoral fellow at The Rockefeller University with the late Hidesaburo Hanafusa.

The August lab is focused upon infection-based and environment signals that trigger inflammation. They have a specific interest in the Tec family of tyrosine kinases and their role in the immune system. With 140 publications to date, he and his laboratory have made seminal contributions to our understanding of how T cells induce and regulate both pro- and anti-inflammatory cytokine production, including interferon gamma (IFNγ), interleukin 17 (IL-17), and interleukin 10 (IL-10). Other interests include how the Tec family of kinases impact the regulation of $CD8^+$ T cell memory development.

Program Schedule

7:30 – 8:00am: Arrival / Check-in

8:00 – 8:30am: Continental Breakfast

8:30 – 10:00am: Oral Session I
Moderator: Nisha Kamath

8:30 Priyanka Rana, Ph.D. (#48)
Immunoproteasome activation expands the MHC Class I antigenic landscape, unmasks neoantigens and reduces tumor growth

8:45 Kevin Newhall (#40)
Specific macrophage states and select Debaryomyces hansenii *strains promote immune evasion*

9:00 Emily Kukan (#30)
M2 macrophages exhibit enhanced environmental sensing of sialylated glycans via the inhibitory receptor CD22

9:15 Brendan Boylan (#6)
Signals driving stromal cell activation factors during CNS inflammation

9:30 Angela DeTomaso (#11)
IL-1β induces a local progesterone withdrawal at the maternal-fetal interface to trigger parturition

9:45 J. Michael Stolley, Ph.D. (#54)
Oral resident memory T cell immunosurveillance of taste buds

10:00 – 10:30am: Poster Flash Talks
Moderator: Brianna Busscher

10:00 Paul Karell (#23)
Rapid attrition is a common feature of IgA responses within the intestine post-antibiotic treatment

10:02 Leandre Glendenning (#15)
Lipopolysaccharide is a regulator of IgG sialylation in endothelial cells

10:04 Ananya Vadlakonda (#59)
Elucidating inflammatory mechanisms involved in the IMQ-induced psoriasis mouse model: A study on the role of isostearic acid in skin inflammation

10:06 Amber Boulton, Ph.D. (#5)
CD6 suppresses CD4 T cell activation and follicular helper cell differentiation during murine coronavirus infection

10:08 Corynn Appolonia (#1)
MicroRNA146a deficiency protects from spontaneous autoimmune diabetes in NOD mice

10:10 James Ignatz-Hoover, M.D., Ph.D. (#20)
Vactosertib, a TGFβ type I receptor/ALK4 kinase inhibitor, improves T-cell fitness: a phase 1b trial in relapsed/refractory multiple myeloma

10:12 Yeojung Koh (#28)
Inhibiting 15-PGDH prevents blood-brain barrier deterioration and blocks neurodegeneration in Alzheimer's disease

10:14 Sofia Corella (#9)
The influence of traumatic brain injury on the hematopoietic system and neuroimmune interactions

10:16 Megan Grund, Ph.D. (#17)
Pathogen Burkholderia cenocepacia

10:18 Daniel Kingsley (#25)
VCAM1-expressing osteosarcoma cells increase metastatic potential through VCAM1-VLA4 signaling axis in macrophages

10:20 Erik Koritzinsky (#29)
mtDNA release and TLR9 signaling enhance ischemia-reperfusion injury and costimulatory blockade resistant memory T cell activation in high-risk allografts

10:30 – 10:45am: Coffee Break

10:45 – 11:45am: Poster Session I

Abstract #	Presenter	Title
1	**Corynn Appolonia**	MicroRNA146a deficiency protects from spontaneous autoimmune diabetes in NOD mice
3	**Meera Bambroo**	*In vitro* manipulation of novel cell lines derived from intestinal epithelial stem cells
7	**Alyssia Broncano**	Sex-specific estrogen signaling modulates T cell responsiveness to TGFβ
9	**Sofia Corella**	The influence of traumatic brain injury on the hematopoietic system and neuroimmune interactions
15	**Leandre Glendenning**	Lipopolysaccharide is a regulator of IgG sialylation in endothelial cells
19	**Alyssa Hubal**	Inhibition of Src signaling induces PERK-mediated selective autophagic killing of *Toxoplasma gondii*
21	**Jacob Ingber**	Insights into heterologous immunity: ELISpot analysis of diverse mouse strains

24	**Emily Katoni**	O-GlcNAcylated NF-κB c-Rel is a transcriptional regulator of acute myeloid leukemia
27	**Bailey Klein**	Mast cell derived histamine is a negative regulator of hematopoietic stem cell function
29	**Erik Koritzinsky**	mtDNA release and TLR9 signaling enhance ischemia-reperfusion injury and costimulatory blockade resistant memory T cell activation in high-risk allografts
32	**Alexander Kuo**	B cell homeostasis and autoantibody responses are tuned by a novel functional interaction between Ezrin and Myo18A
33	**Kelsey Lewis**	Mechanisms of immunomodulation of blueberries in intestinal inflammation and injury
36	**Megan Long**	Regulation of IgG sialylation by cytokine mediators
37	**Dajana Margeta**	Spatial transcriptomics reveal the Achilles heel of glomeruli in antibody mediated rejection
38	**Sarah McNeer**	Nuclear estrogen receptors modulate T cell pathogenicity in an *in vivo* colitis model
42	**Katelyn O'Hare**	Cholesterol biosynthesis pathways in monocyte-derived macrophages link cytomegalovirus infection to atherosclerosis
43	**Meghan O'Keefe**	Inflammasome activation drives parallel signaling pathway inactivation
44	**Erica Orsini**	The role of macrophage Piezo1 on bacterial clearance in cystic fibrosis
47	**Lane Pierson**	Development of cellular assays to characterize the impact of sequence variation on STING function in bats
50	**Vanessa Salazar**	Skin microbiota drive checkpoint inhibitor toxicity in a genetic autoimmune susceptible mouse
51	**Avinaash Sandhu**	Determining the genes and pathways in M2 macrophages responsible for subverting CD4+ T-cell activation upon infection with *Mycobacterium tuberculosis*
57	**Reyhaneh Tabatabaei**	Development of vaccines with adjuvant in patients with metabolic diseases
58	**Vinayak Uppin**	CAR-T cell targeting three receptors on autoreactive B cells for systemic lupus erythematosus therapy
60	**Kaylynn Vidmar**	*Candida tropicalis* infection during DSS colitis induces expansion of IL-1β and IL-23-producing NOD2-expressing macrophages
61	**Hannah Wargo**	Microbial influence on sex-based differences in a Crohn's disease-like ileitis model

63	**Joseph Williams**	Gasdermin B (GSDMB) associates with –and regulates– GSDMD in intestinal-derived goblet cells and is upregulated in inflamed tissues of ulcerative colitis (UC) patients

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

1:00 – 2:30pm: Oral Session II
Moderator: Erik Koritzinsky

1:00 Huong Nguyen, Ph.D. (#41)
TME-gated inducible CAR (TME-iCAR) T-cell therapy for solid tumors

1:15 Anna Winnicki (#64)
Potent AMA1-specific human monoclonal antibody against Plasmodium vivax pre-erythrocytic and blood stages

1:30 Vinicius Suzart (#56)
Human CD4+ T cells recognize autologous bystander non-infected macrophages exposed to soluble Mycobacterium tuberculosis antigens but lack IFNγ production

1:45 Angela Liu (#35)
Novel inflammatory mechanism in psoriasis pathogenesis: NF-κB c-Rel regulates TLR7 signaling in dendritic cells

2:00 Robert Schauner (#52)
Single cell and bulk RNA expression analyses identify enhanced hexosamine biosynthetic pathway and O-GlcNAcylation in acute myeloid leukemia blasts and stem cells

2:15 Christina Farr Zuend, Ph.D. (#12)
The DIVA study: Defining the neovaginal microenvironment

2:30 – 2:45pm: Coffee Break

2:45 – 3:45pm: Poster Session II

Abstract #	Presenter	Title
2	**Pari Baker**	Exploring maternal antibody dynamics: Insights from the ferret animal model
4	**Adam Boulton**	A novel TRPV4-NF-κB/p65 inhibitory interaction to regulate macrophage pro-inflammatory function
5	**Amber Boulton**	CD6 suppresses CD4 T cell activation and follicular helper cell differentiation during murine coronavirus infection
8	**Brianna Busscher**	The β-propeller domain of VPS39 protein is critical to its interaction with SARS-CoV-2 ORF3a

10	**Jordan Cress**	WNK1 is a novel regulator of acute myeloid leukemia
13	**Daniel Feinberg**	Inhibition of O-GlcNAcylation disrupts COPII vesicle formation in NK cells
14	**Cheyanne Foster**	Unraveling unconventional pathways: NLRP3 inflammasome signaling and IL-1β release in murine bone marrow derived mast cells
16	**Zachary Grimmett**	Metabolic reprogramming by the denitrosylase S-nitroso-Coenzyme A Reductase 2
17	**Megan Grund**	Pathogen *Burkholderia cenocepacia*
18	**D'Atra Hill**	Investigating mucosal neutrophils in HPV-associated cervical neoplasia in African American women
20	**James Ignatz-Hoover**	Vactosertib, a TGFβ type I receptor/ALK4 kinase inhibitor, improves T cell fitness: a phase 1b trial in relapsed/refractory multiple myeloma
22	**Nisha Kamath**	Large-scale functional characterization of the STIM1 cEFhand reveals residues important for calcium influx and immune response
23	**Paul Karell**	Rapid attrition is a common feature of IgA responses within the intestine post-antibiotic treatment
25	**Daniel Kingsley**	VCAM1-expressing osteosarcoma cells increase metastatic potential through VCAM1-VLA4 signaling axis in macrophages
26	**Riley Kirkpatrick**	Identification of novel biomarkers of immune toxicity from CAR-T cell therapy using ultrasensitive NULISA™ proteome technology
28	**Yeojung Koh**	Inhibiting 15-PGDH prevents blood-brain barrier deterioration and blocks neurodegeneration in Alzheimer's disease
31	**Nikhil Kulkarni**	Cervical immunity is altered in Canadian women who have bacterial vaginosis
34	**Feng Lin**	A nanobody-based complement inhibitor targeting complement Component 2 (C2) reduces hemolysis in a complement humanized mouse model of autoimmune hemolytic anemia
39	**Sekinat Mumuney**	Assessing antibody-bound microbes in the female reproductive tract
45	**Anne-Marie Overstreet**	A novel protective role for Hmgb1 in the gastrointestinal lumen
46	**Michelle Pan**	Induction of necroptosis releases MLKL's executioner domain before its RIPK3-mediated phosphorylation
49	**Bibekananda Sahoo**	The mechanism of plasma membrane rupture at the end of pyroptosis and necroptosis

53	**Sadiq Silbak**	Epitope mapping of the α-FXIIa allosteric inhibitor monoclonal antibody 5C12
55	**Xi Su**	CD57+ T cell transmigration through vascular endothelial cells is enhanced by TNF: A novel model of cardiovascular risk in people with HIV
59	**Ananya Vadlakonda**	Elucidating inflammatory mechanisms involved in the IMQ-induced psoriasis mouse model: A study on the role of isostearic acid in skin inflammation
62	**Quentin Watson**	Evaluating the invasion of erythroid lineage cells by *Plasmodium vivax* and *Plasmodium knowlesi* (PvDBPOR) transgenic parasites

4:00 – 5:00pm: **Ecker Memorial Lecture**
Dr. Avery August, Ph.D.
HHMI Professor and Professor of Immunology
Department of Microbiology and Immunology
Cornell University School of Veterinary Medicine

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

5:30 – 6:30pm: **Reception**

Abstracts

1

MicroRNA146a deficiency protects from spontaneous autoimmune diabetes in NOD mice

Corynn Appolonia[1], Shrikanth Basavarajappa[1], Joshua Centore[1], and Parameswaran Ramakrishnan[1]

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

Type 1 diabetes (T1D) is a chronic autoimmune disease that results in destruction of pancreatic islet beta cells. We have previously shown that deficiency of NF-kB c-Rel, the key transcription factor that controls FOXP3 expression and T regulatory (Treg) cell development and function, accelerates spontaneous autoimmune diabetes in non-obese diabetic (NOD) mice. Interestingly, c-Rel knockout NOD mice showed significantly increased levels of microRNA146a (miR146a), which is involved in post-transcriptional suppression of key NF-κB signaling proteins. Similarly, miR146a is increased in the serum of T1D patients at the time of diagnosis. Hence, we generated miR146a-knockout (miR146a-KO) NOD mice to study the role of miR146a in T1D. We found that deficiency of miR146a significantly protected NOD mice from spontaneous autoimmune diabetes. While we found no impact of miR146a deficiency on general hematopoietic parameters and immune cell populations, remarkably, immune cell infiltration into the pancreas was significantly inhibited. MicroRNA146a-KO mice were found to have increased Treg cells with greater suppressor function, and miR146a-KO Treg cells have increased NF-kB c-Rel protein, which is a likely mechanism for the protection from T1D. This study reveals a previously unknown role of miR146a in the pathogenesis of autoimmune diabetes and sets the stage for translational studies targeting miR146a in T1D and other autoimmune diseases.

2

Exploring maternal antibody dynamics: Insights from the ferret animal model

Pari Baker[1], Claire Vercruysse[1], Michelle Moyer[1], Stephanie N. Langel[1]

[1] Center for Global Health and Diseases, Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH, USA

Maternal antibodies (matAbs) passively transfer via the placenta and breast milk and are critical for protection against influenza disease in neonates. However, high concentrations of matAbs in infant circulation can impact de novo immune responses to immunization, known as matAb interference. It is unknown what levels of matAbs limit neonatal immune responses after influenza vaccination. To better understand the mechanisms of matAb interference, we sought to develop a ferret model of IgG passive transfer to neonates. First, we developed a ferretized IgG1 anti-hemagglutinin stem monoclonal antibody (mAb) that was systemically administered into pregnant ferrets at 1mg/kg or 5mg/kg one-week prepartum. We hypothesized that the ferretized mAb would passively transfer from dams to suckling kits in a dose-dependent manner. Serum was collected from dams and kits at 1, 2, 6 and 9 weeks postpartum and breast milk was collected until weaning to study mAb pharmacokinetics. Samples were assessed via ELISA for the presence of HA-specific IgG antibodies. We detected mAb concentrations in both dam milk and serum at each timepoint and identified peak concentrations of mAb in kit serum at 1 week postpartum ($P < 0.0001$). Surprisingly, we identified no dose-dependent differences between kits from dams of either treatment group at any timepoint ($p \geq 0.86$). Our current data suggests that mAb is passively transferred to kits via breast milk, reaching peak concentrations in kits one week postpartum. This model will be used in future work to assess the impact of matAbs on neonatal vaccine responses and protection against influenza challenge.

3

In vitro manipulation of novel cell lines derived from intestinal epithelial stem cells

Meera Bambroo[1,2], Julie Zhou[2], Qiuhe Lu[2], Thaddeus Stappenbeck[2]

[1] Department of Biology, Case Western Reserve University

[2] Department of Inflammation & Immunity, Cleveland Clinic Lerner Research Institute

The intestinal epithelium serves as the primary defense against luminal contents by establishing a continuous barrier with tight and adherent junctions between epithelial cells. In metastatic colorectal cancer, epithelial cells can undergo a process called epithelial-to-mesenchymal transition (EMT) and lose the expression of proteins that maintain these junctions, including ZO-1 and E-Cadherin. Here, we modeled this process using novel immortalized intestinal epithelial cell (iIEC) lines derived from mouse jejunal and colonic epithelial stem cells that were adapted to grow in a 2D format in complete DMEM. Compared to the primary cells that they were immortalized from, iIECs express fewer epithelial cell features and more mesenchymal cell features. We tested strategies to reverse EMT using immunofluorescence staining for junctional proteins paired with confocal microscopy. Supplementation of butyric acid, a microbial product previously shown to promote tight junctional proteins, did not appear to improve their expression. Changing the culture media to one that supports primary intestinal epithelial cell growth also did not. However, pharmacologic inhibition of TGFβ receptor signaling increased the expression of junctional proteins along the epithelial membrane, suggesting the cell lines can undergo EMT reversal and that TGFβ signaling mediates this process. Our findings show that the novel iIEC lines generated in our lab can undergo a reversal of EMT, termed mesenchymal-to-epithelial transition (MET), to improve epithelial phenotype through increased expression of tight junction proteins. Moreover, the results suggest that these cell lines can be used as a model for colorectal cancer cells to further research how EMT in the intestinal epithelium can be mediated.

4

A novel TRPV4-NF-κB/p65 inhibitory interaction to regulate macrophage pro-inflammatory function

Adam M. Boulton[1], M. Grund[1], Y. Wang[1], E. M. Orsini[3], Y. Liu[1], S. Abraham[1], L.M. Grove[1], R. Musich[1], B.D. Southern[1,3], A. Reinhardt[1], G. Stark[2], V. Vachharajani[1,3], M. A. Olman[1,3], R. G. Scheraga[1,3*]

[1] Inflammation and Immunity, Lerner Research Institute, Cleveland Clinic

[2] Cancer Biology, Lerner Research Institute, Cleveland Clinic

[3] Integrated Hospital Care Institute, Department of Pulmonary and Critical Care, Cleveland Clinic

Macrophages are innate immune cells that defend against pulmonary infections, maintaining lung homeostasis. In acute respiratory distress syndrome (ARDS) recruited lung macrophages are plastic, both causing acute lung injury through secretion of pro-inflammatory cytokines for pathogen clearance, and essential for lung injury repair. Macrophage activation and pathogen response requires a soluble and mechanical signal (e.g. lipopolysaccharide (LPS) and matrix stiffness). We have shown that Transient Receptor Potential Vanilloid 4 (TRPV4) a mechanosensitive-cation channel is critical for limiting pro-inflammatory cytokine secretion and lung injury by macrophages during bacterial pneumonia. NF-κB/p65 is a transcription factor complex contributing to a pro-inflammatory response by increasing cell proliferation, angiogenesis, and cytokine production. Here, we show that a TRPV4 interaction regulates the NF-κB/p65 pathway. Utilizing scRNA-seq, we found bone marrow derived macrophages (BMDMs) from TRPV4 knockout mice have enrichment of NF-KB pro-inflammatory genes vs WT BMDMs in both basal and LPS-treated conditions. Additionally, loss of TRPV4 function enhanced IL-1β secretion in murine and human ARDS-derived macrophages. This effect was blocked by downregulation of the NF-κB pathway (p65-siRNA), suggesting TRPV4 mediates a phenotypic shift in macrophages. Direct biochemical interaction of TRPV4 and NF-κB pathway proteins was demonstrable by co-immunoprecipitation and biomolecular fluorescence complementation. Using homology modeling and in silico molecular dynamic simulations we show that the TRPV4 N-terminal domain interacts with NF-κB transcription factor p65. Given that NF-κB/p65 plays such a crucial role in a variety of cell types, identifying a macrophage specific regulator of NF-κB action has multiple implications in lung injury/ARDS.

5

CD6 suppresses CD4 T cell activation and follicular helper cell differentiation during murine coronavirus infection

Amber C. Boulton[1], Feng Lin[2], and Cornelia Bergmann[1]

[1] Department of Neuroscience, Cleveland Clinic Lerner Research Institute

[2] Department of Inflammation and Immunity, Cleveland Clinic Lerner Research Institute

CD6 is a surface glycoprotein predominantly expressed on T cells that can function as both a co-stimulatory and co-inhibitory receptor during T cell activation. During experimental autoimmune encephalitis CD6 functions as a T cell co-stimulatory receptor that is critical for pathogenic T cell activation, survival, and infiltration of the central nervous system. This study therefore sought to determine how CD6 regulates T cell activation after viral infection of the central nervous system. Infection of CD6 deficient mice with a murine neurotropic coronavirus resulted in increased CD4 T cell activation and expansion in the draining cervical lymph nodes. The expanded CD4 T cell response also displayed accelerated and preferential differentiation into CD4 T follicular helper cell differentiation. Consequently, B cell activation, expansion, differentiation into germinal center cells, and high-affinity virus-specific antibody production was also accelerated and enhanced in CD6 deficient mice. Investigation into the enhanced CD4 T cell response revealed that upregulation of the CD6 binding protein *Ubash3a*, an established suppressor of T cell receptor signaling, was significantly hampered in CD6 deficient lymph nodes. Consistent with impaired UBASH3a activity, CD6 deficient CD4 T cells had stronger T cell receptor signaling. These results reveal a novel role for CD6 in negatively regulating T cell activation, differentiation, and anti-viral humoral immunity, indicating that CD6 might be a target to enhance vaccine induced antibody responses.

6

Signals driving stromal cell activation factors during CNS inflammation

Brendan T. Boylan[1], Mihyun Hwang[1], Elyse Brozost[1], Hugh Oh[1], Antoine Louveau[1], Cornelia Bergmann[1]

[1] Department of Neuroscience, Cleveland Clinic Lerner Research Institute

Chemokines are essential for recruitment of peripheral immune cells to the central nervous system (CNS) during infection, trauma, and autoimmunity. CNS stromal cells, especially perivascular fibroblasts, and endothelial cells, play prominent roles in supporting leukocyte accumulation. Chronically activated meningeal and perivascular fibroblasts releasing chemokines can drive pathogenic immune cell aggregates known as tertiary lymphoid organs (TLOs) during human neuroborreliosis and multiple sclerosis (MS). Initial activation of these cells may lay the groundwork for future unremitting activation which could promote TLOs, but the cells and signals driving this initial activation are unexplored. Here we use the neurotropic murine hepatitis virus (MHV) infection model to study early activation of chemokine producing fibroblasts. IgD+ B cells appear in the CNS during several inflammatory models suggesting they may play innate-like roles to activate CNS fibroblasts through lymphotoxin β (LTβ), a molecule known to signal to fibroblasts in the periphery. Using inducible B cell specific knockout mice and LTβ agonism, we found that B cells and LTβ contribute to chemokine induction, immune cell recruitment, and viral control. Further assessment of a role for pattern recognition receptor mediated activation using toll-like receptor agonists and knockout mice revealed that CCL19 was dependent on Myd88, but podoplanin, a marker of fibroblast activation, was independent of Myd88. Overall, our data indicate that stromal cells integrate direct cell intrinsic and indirect immune signals to tune their activation for effective antiviral responses.

7

Sex-specific estrogen signaling modulates T cell responsiveness to TGFβ

Alyssia V. Broncano[1], Sarah K. McNeer[1], William B. Tran[1], Adrian D. Kocinski[1], Wendy A. Goodman[1]

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

TGFβ signaling is required for numerous T cell functions, including differentiation of naïve T cells into regulatory T cells (Tregs) and Th17 cells. Previous studies have shown that signaling via 17β-estradiol (estrogen, E2) through its nuclear receptors alpha and beta (ERα and ERβ) can modulate TGFβ signaling in cancer cell lines. We previously showed that reduced expression of ERβ in naïve $CD4^+$ T cells inhibits induction of Foxp3 in response to TGFβ signaling, thus preventing Treg differentiation. This led us to hypothesize that E2 signaling impacts the responsiveness of primary $CD4^+$ T cells to TGFβ signaling. To test this, we isolated $CD4^+$ T cells from spleen and lymph nodes of male and female wild-type (WT) mice or mice globally deleted for ERα or ERβ (ERα-KO, ERβ-KO) and assessed their responsiveness to TGFβ signaling *ex vivo*. Although expression of TGFβ receptors I and II (TGFβRI, RII) was comparable in bulk $CD4^+$ T cells isolated from all groups, expression of TGFβRI was reduced in naïve T cells ($CD4^+CD44^-CD62L^+$) isolated from female (but not male) mice. In agreement with this, $CD4^+$ T cells isolated from females displayed delayed and less intense phosphorylation of Smad3 in response to TGFβ stimulation compared to cells isolated from males. Taken together, our results suggest that naïve T cells isolated from females are resistant to immunoprotective TGFβ signaling, potentially contributing to enhanced inflammation and incidence of autoimmune disease in females.

8

The β-propeller domain of VPS39 protein is critical to its interaction with SARS-CoV-2 ORF3a

Brianna M. Busscher[1], Tsan Sam Xiao[1]

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

SARS-CoV-2, the virus that causes COVID-19, continues to be a global health concern, and understanding its mechanisms of pathogenesis is crucial to developing new therapeutic approaches. One of the viral accessory proteins, open reading frame 3a (ORF3a), was shown to be important for pathogenesis, potentially driven by the protein's multiple functions. For example, ORF3a has been reported to block autophagy by preventing the fusion of autophagosomes with lysosomes through binding to vacuolar protein sorting 39 (VPS39) protein. VPS39 participates in the HOPS complex, which facilitates membrane fusion between autophagosomes and lysosomes. The interaction between ORF3a and VPS39 has been demonstrated by multiple groups, but the structural interface remains unknown. Co-immunoprecipitation with SARS-CoV-2 ORF3a and human VPS39 revealed that the N-terminal β-propeller domain of VPS39 is sufficient to pull down ORF3a, and the absence of the β-propeller diminishes ORF3a pull down. Mutation of two residues in ORF3a (S171E and W193R) reduced pull down of the VPS39 β-propeller. These data suggest that the VPS39 β-propeller domain is critical to the ORF3a:VPS39 interaction, and that ORF3a residues Ser171 and Trp193 play important roles in this interaction. Structural studies using CryoEM and X-ray crystallography will be pursued to determine the molecular mechanisms of the VPS39 β-propeller binding to ORF3a in suppression of autophagy by SARS-CoV-2.

9

The influence of traumatic brain injury on the hematopoietic system and neuroimmune interactions

Sofia Corella[1,2,3,4], Bailey Klein[1,5], Sarah Barker[1,2,3,4], Edwin Vázquez-Rosa[1,2,3,4], Amar Desai[5], Andrew A. Pieper[1,2,3,4]

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[2] Department of Psychiatry, Case Western Reserve University, Cleveland, OH
[3] Brain Health Medicines Center, Harrington Discovery Institute, University Hospitals, Cleveland, OH
[4] Geriatric Research Education and Clinical Center (GRECC), Louis Stokes Cleveland VA Medical Center, OH
[5] Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH

Traumatic brain injury (TBI) is associated with an increased risk of developing dementia, including Alzheimer's disease. A proposed mechanistic link between TBI and dementia is harmful, chronic neuroinflammation. Although extensive research on neuroimmune interactions within and outside the CNS implicate the immune system in mediating neurodegeneration, our understanding of the interactions between the nervous and hematopoietic systems in TBI remains limited. The hematopoietic system is composed of hematopoietic stem and progenitor cells residing within specialized microenvironments in bone marrow that promote hematopoietic stem cell (HSC) self-renewal, as well as differentiation and maturation. Despite the role of the hematopoietic system in guiding development and functionality of the immune response to TBI, the impact of TBI on the hematopoietic system, and the extent of such impact on the immune system and neurological health, has yet to be well characterized. Here, we employed the clinically relevant murine model of multimodal TBI (mmTBI), which mimics key aspects of human TBI, including blood-brain-barrier deterioration, brain tau pathology and blood acetylated-tau accumulation, axonal degeneration, and cognitive impairment. Using sham-TBI and mmTBI bone marrow, we performed long-term and serial bone marrow transplantations in irradiated, uninjured mice. Notably, we show impaired ability of TBI-derived HSCs to engraft, self-renew, reconstitute the hematopoietic system, and give rise to blood cells in recipient mice. In mmTBI animals, we also show aberrant immune cell numbers and dysregulated inflammatory mediators, suggesting a role for TBI-induced hematopoietic system dysfunction in driving immune system deficits and promoting pathologic features of TBI, such as chronic neurodegeneration.

10

WNK1 is a novel regulator of acute myeloid leukemia

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Acute Myeloid Leukemia (AML) is the transformation of immature myeloid blasts which prevents differentiation and causes hyperproliferation. Treatments such as induction chemotherapy and All-trans-retinoic acid (ATRA) have improved overall survival. However, the success of these treatments is limited, and around 60-70% of patients will die within 5 years of diagnosis. Thus, there is a need to further characterize mechanisms that drive AML abnormalities. A unique characteristic that distinguishes AML cells from normal myeloid progenitors is altered metabolism. This has primarily been characterized as increased glycolysis and glucose uptake. We have also shown that aberrant AML metabolism includes elevated activity of the Hexosamine Biosynthetic Pathway (HBP). The HBP produces UDP-GlcNAc which can then be added onto nuclear and cytoplasmic proteins in a process called O-GlcNAcylation. Previous work has shown that inhibiting total O-GlcNAcylation causes AML apoptosis, however, it remains unclear how these effects are mediated and how specific O-GlcNAcylated proteins may contribute. We identified With-no-Lysine(K) kinase 1 (WNK1) as a prominent O-GlcNAcylation target in AML. We found that inhibiting WNK1 kinase function resulted in decreased viability and cell cycle progression of AML cells. In addition, inhibiting the well-established substrates of WNK1: Ste20-related proline-alanine-rich kinase (SPAK) and Oxidative stress responsive kinase 1 (OSR1), caused similar effects. Inhibiting WNK1 also induced AML cell differentiation which was further enhanced by combination treatment with ATRA. Finally, we demonstrate that WNK1O-GlcNAcylation enhances its stability. Taken together, our findings implicate a role for WNK1 signaling and its O-GlcNAcylation in promoting AML survival, growth, and dedifferentiation.

11

IL-1β Induces a local progesterone withdrawal at the maternal-fetal interface to trigger parturition

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Preterm (< 37 completed weeks of gestation) birth occurs in 10-15% of pregnancies and causes majority of neonatal mortality and morbidity. Pregnancy is maintained by modulation of the maternal immune system at the maternal-fetal interface (MFI) to prevent immunologic rejection of the semi-allogeneic conceptus. This process is thought to be controlled by the steroid hormone progesterone (P4), acting via its nuclear P4 receptor (PR). Loss of P4/PR signaling at the MFI promotes tissue-level inflammation and activates the parturition cascade; however, our data suggest that inflammation itself reduces P4/PR activity. This is important because inflammation is a major causal factor in the control of both term and preterm birth. A potential mechanism by which inflammation reduces P4 responsiveness at the MFI is via the metabolism of P4 to an inactive form by the aldo-keto reductase family 1 member C1 (AKR1C1) enzyme. In Rhesus macaques and mice, intra-uterine infection induced preterm labor and increased AKR1C1 expression in the MFI. In human MFI, AKR1C1 mRNA increased in association with labor. In human MFI explants and a human decidual stromal cell line (iDEC), IL-1b (a pro-labor cytokine) increased abundance of mRNA encoding AKR1C1 while an AKR1C1 inhibitor repressed IL-1β responsiveness, and prevented LPS-induced preterm labor in mice, consistent with preserved P4/PR anti-inflammatory activity. Moreover, IL-1β inhibited P4-induced expression of IL15 and this was prevented by R5020 (a non-metabolizable progestin). Finally, P38 is a key mediator of IL-1β-induced AKR1C1 expression and P4 withdrawal in decidual stromal cells.

12

The DIVA study: Defining the neovaginal microenvironment

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Gender affirming care for transgender women can include vaginoplasty, which is the surgical creation of a neovagina using penile inversion, sigmoid colon grafts, or peritoneal flaps. Limited data exists describing the neovaginal microenvironment, which is vital for providing evidence-based care to people with neovaginas. We have established a cohort of transgender women that are at least 3 months post vaginoplasty to characterize the neovaginal microenvironment. Participants provide a variety of biospecimens and complete an extensive questionnaire including their sexual history and behaviors, diet, surgical satisfaction (SSQ-8), and gender dysphoria. Neovaginal samples will be analyzed using a multi-'omics approach including 16S rRNA sequencing and cytokine array. There are 33 transgender women currently enrolled in the DIVA cohort. The median age is 37 (range 24-68), with a median time since vaginoplasty of 404 days (range 97-2,205 days). Nineteen (57.6%) participants are currently using hormone therapies. Nineteen (57.6%) participants have had additional surgery since primary vaginoplasty including for vaginal stenosis or aesthetic purposes. The majority of participants (21, 63.6%) report at least 1 neovaginal symptom in the past month, including discharge (10, 30.3%), pain (8, 24.2%), or odor (6, 18.2%). Five (15.5%) participants report having neovaginal intercourse in the past month. Participant recruitment and biospecimen analysis is ongoing. We have established the DIVA study to characterize the neovaginal micro-environment. The results of this study will provide information on the neovaginal microbiome, genital inflammation, and relationship to gynecological health, which is critical for post-operative and long-term neovaginal care.

13

Inhibition of O-GlcNAcylation disrupts COPII vesicle formation in NK cells

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O-GlcNAcylation is a post-translational modification where N-acetylglucosamine (GlcNAc) is added to serine and threonine residues of proteins. O-GlcNAc transferase (OGT) adds the monosaccharide to the amino acid and O-GlcNAcase (OGA) removes it. This process is highly evolutionarily conserved, and often necessary for organism/cellular survival, including T-cell survival. Yet, the role of O-GlcNAcylation in primary natural killer (NK) lymphocyte function remains largely unexplored. Our recent publication showed that OGT inhibition results in decreased cytotoxicity and inhibition of cytotoxic mediator secretion in NK cells. Here, we set out to explore if decreased O-GlcNAcylation is disrupting COPII vesicle (composed of Sec13/23/24/31) localization in primary NK cells. Our mass spectrometry of O-GlcNAcylated proteins showed Sec13 (a heterodimeric partner of Sec31) as a significant hit. Additionally, we show Sec13/31 are present in NK cells by western blot unaffected by OGT inhibition. We show that inhibition of COPII vesicle formation disrupts NK cell cytotoxicity/secretion in the same pattern as inhibition of O-GlcNAcylation (perforin most affected and IL-17 unaffected).Finally, we show that Sec13/31's localization is disrupted when OGT is chemically inhibited as analyzed by AMNIS. This is a first-of-its-kind use of AMNIS. Taken together, we show decreased O-GlcNAcylation inhibits COPII vesicle localization in NK cells disrupting secretion and inhibiting cytotoxicity.

14

Unraveling unconventional pathways: NLRP3 inflammasome signaling and IL-1β release in murine bone marrow derived mast cells

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Activation of the NLRP3 inflammasome involves hierarchical activation and assembly of upstream protein complexes, ultimately releasing IL-1β – a potent proinflammatory cytokine that is implicated in a variety of physiological and pathological responses. Previous studies utilize macrophages as a model system for interrogating the NLRP3 inflammasome, and many MC studies extrapolate these data onto MCs. However, our studies indicate that murine bone marrow derived mast cell (BMMC) NLRP3 inflammasome signaling and IL-1β release follows a different mechanism to those previously studied in macrophages. Preliminary data using BMMCs suggests that IL-1β is released in a GSDM-independent manner. Our MC studies have demonstrated a phenomenon where NLRP3 priming by IL-33 is more efficacious than the TLR4-dependent pathway activated by LPS. Additionally, we observed differences in efficacy between the ability of ATP and Nigericin to serve as "signal 2" agonists in the release of IL-1β, indicating a difference in how BMMCs translate K^+ efflux signals to inflammasome assembly. These differences in efficacy track with the ability of ATP to serve as a degranulation stimulus, indicating functional links between degranulation and NLRP3 activity. We seek to unravel the involvement of degranulation processes in facilitating IL-1β export, paving the way for a deeper understanding of mast cell biology, and potentially uncovering novel targets for therapeutic intervention in inflammatory disorders.

15

Lipopolysaccharide is a regulator of IgG sialylation in endothelial cells

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Sialylation of the conserved glycan on the Fc domain of IgG comprises a key “inflammatory switch” in the host immune response. For decades, it has been well established that decreases in IgG Fc sialylation strongly correlate with various inflammatory conditions, ranging from infections such as HIV and tuberculosis to chronic autoimmune disorders such as rheumatoid arthritis. Despite the abundance of evidence that IgG sialylation is tightly regulated in disease pathogenesis, the mechanisms underlying the transition from asialylated to sialylated IgG remain uncertain. Conventionally, it has been presumed that IgG sialylation occurs in the trans-Golgi network of antibody-producing plasma cells; however, we demonstrated in 2016 that mice lacking the sialyltransferase ST6Gal1 in the B cell compartment have unaltered plasma IgG sialylation. Further, we have shown that B cells produce largely asialylated IgG, leading us to conclude that IgG sialylation is largely regulated following its secretion into the plasma by the B cells. Here, we report that isolated endothelial cells are capable of sialylating IgG in the culture supernatant. Additionally, we demonstrate that lipopolysaccharide isolated from the bacterial outer membrane downregulates expression of ST6Gal1 in endothelial cells and lowers their ability to sialylate IgG. Together, these data support the existence of a glycan remodeling pathway in endothelial cells that is regulated by antigen exposure and drives B cell-extrinsic IgG sialylation.

16

Metabolic reprogramming by the denitrosylase S-nitroso-Coenzyme A Reductase 2

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All cells respond to external or internal stimuli by regulating their metabolic pathways to match current conditions, and these changes can be manipulated for therapeutic gain. This dynamic metabolic reprogramming is tightly regulated, and acute regulation classically relies on protein post-translational modifications. One of these is S-nitrosylation, the formation of S-nitrosothiol (SNO; derived from nitric oxide) on cysteine residues. Protein S-nitrosylation is regulated by specific enzymes that form (nitrosylases) and degrade (denitrosylases) the SNO moiety, respectively. SNO-CoA Reductase 2 (SCoR2; product of the Akr1a1 gene) is a recently discovered, widely expressed protein denitrosylase that has been shown to regulate a metabolic response to kidney injury, but its roles in other organs such as the heart remain unknown. Here we show that SCoR2 regulates the S-nitrosylation state and activity of multiple metabolic proteins and pathways that control ketolytic energy availability, antioxidant levels, and harmful polyol compounds in mouse plasma and heart. As a consequence, mice lacking SCoR2 exhibit robust cardioprotection in an ischemia-reperfusion model. Widespread, coordinated reprogramming of multiple metabolic pathways through elevated S-nitrosylation of specific metabolic enzymes resulting from SCoR2 inhibition thereby limits infarct size to confer ischemic cardioprotection. Because of its widespread expression, SCoR2 thus appears likely to serve as a master metabolic regulator in heart, kidney, immune lineages, and cancer cells.

17

TRPV4-Dependent Bacterial Clearance of Cystic Fibrosis Pathogen *Burkholderia cenocepacia*

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Cystic Fibrosis (CF) is an immunocompromising disease caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. Defects or deletions of the CFTR protein affect mucus composition, decreasing pulmonary function and infection clearance. Current CFTR-based therapeutics modulate protein production or function, but do not address the common and lethal infections from bacteria. *Burkholderia cenocepacia* is a chronic antibiotic-resistant CF pathogen with a 40-75% mortality rate. Due to the bacteria's intracellular lifestyle and genetic plasticity, therapeutic options are currently limited to select antibiotics. Here, we aim to identify mechanisms of lung host response to B. cenocepacia. Our lab previously identified transient receptor potential vanilloid 4 channel (TRPV4) in pulmonary macrophages is required for bacteria-induced cytokine secretion and phagocytosis. Currently, it is unknown if TRPV4 is necessary for clearance of *B. cenocepacia*. Here, we show in an *in vivo* model of acute *B. cenocepacia* infection that C57Bl/6 mice have a significant decrease in pulmonary bacterial burden compared to *TRPV4*$^{-/-}$ mice. We also observed a trend towards increased pulmonary cytokine/chemokine profiles (IL-23, MCP-1, MIP-3, MIG, MIP1α, MIP1β) in WT to *TRPV4*$^{-/-}$ mice, suggesting a functional role for TRPV4 in *B. cenocepacia* infections. Additionally, we report female mice, regardless of genotype, had increased bacterial burden and inflammatory markers compared to males. Future studies will continue to discern the functional role of TRPV4 in CFTR-deficient cells and identify potential pathways to therapeutically target CF infections.

18

Investigating mucosal neutrophils in HPV-associated cervical neoplasia in African American women

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Cervical cancer is a common cancer in women, causing over 300,000 deaths each year, with African-American women disproportionately affected. High-risk human papillomavirus (HR-HPV) is a significant cause of cervical cancer and the precursor lesions cervical intraepithelial neoplasia (CIN). Increased neutrophil density and cervical inflammation has been linked to high-grade lesions and cervical cancer progression, but the molecular mechanisms are poorly understood. We have undertaken an analysis of cervical neutrophils collected from women undergoing colposcopy for abnormal PAP tests as part of a larger study of mucosal mechanisms of HPV clearance (THRIVE-HPV) at University Hospitals Cleveland Medical Center. Neutrophils isolated from cervical cytobrushes were immunophenotyped by flow cytometry, and the microbiome was profiled using 16S rRNA sequencing. Histology results identified 9 (37.5%) women with neoplasia, 5 (20.8%) high-grade (CIN2/3), 3 (12.5%) low-grade (CIN1), and 1 indeterminant. An average of 15.8% of live cells detected by flow cytometry were CD66b+ neutrophils (range 0.06-71.8%), and most were activated (CD11b+CD62L-/dim; mean 52.9%, range 7.4-88.9%). In preliminary microbiome analysis, *Lactobacillus* was the most abundant genera detected, followed by *Gardnerella, Streptococcus, Lachnospiraceae, and Megasphaera.* CD66b+ neutrophils were negatively correlated with *Lactobacillus* (r=-0.515, p=0.044) and positively correlated with dysbiotic bacteria including *Gardnerella* (r=0.514, p=0.044), *Atopobium* (r=0.612, p=0.014), and *Megasphaera* (r=0.559, p=0.026). Data demonstrates that cervical neutrophils are abundant in cytobrush samples and positively correlate with bacterial genera that are associated with vaginal microbial dysbiosis. Future studies will include in-depth phenotyping of neutrophils and relate these findings to HPV, CIN, and the progression of cervical neoplasia.

19

Inhibition of Src signaling induces PERK-mediated selective autophagic killing of *Toxoplasma gondii*

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Toxoplasma gondii, an obligate intracellular protozoan parasite, chronically infects approximately one-third of the global population, leading to severe ocular and cerebral manifestations in both immunocompromised and immunocompetent patients. *T. gondii* evades autophagic targeting, a critical host defense mechanism, by manipulating signaling pathways that promote autophagy. *T. gondii* can prevent autophagic targeting by activating Akt, a negative regulator of autophagy, through both Src-mediated EGFR-dependent and EGFR-independent pathways. Src inhibition promotes the formation of the autophagosome, facilitating parasite killing. However, the origin of the selective autophagosome following Src inhibition is unclear. Genetic or pharmacological inhibition of Src disrupts *T. gondii*-induced Src phosphorylation while upregulating PERK, an unfolded protein response sensor and autophagy stimulator. Interestingly, upon parasite invasion into host cells, the endoplasmic reticulum (ER) quickly surrounds the *T. gondii* parasitophorous vacuole, highlighting intricate host-parasite membrane interactions. Thus, we hypothesize that inhibition of Src induces PERK-mediated selective autophagy originating from the ER. Inhibition of Src activates PERK and downstream autophagy mediators, including AMPK and ULK1. PERK- and AMPK-dependent killing of *T. gondii* through selective autophagy is evident from the failure to recruit autophagy and lysosomal markers (LC3 and LAMP-1, respectively) or promote killing in knockdown cells. In addition, immunofluorescence and electron microscopy reveal the recruitment of autophagy mediators to the ER surrounding the parasite. In conclusion, PERK appears to be a key mediator of anti-*T. gondii* selective autophagic targeting following Src activation.

20

Vactosertib, a TGFβ type I receptor/ALK4 kinase inhibitor, improves T-cell fitness: a phase 1b trial in relapsed/refractory multiple myeloma

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Immune escape is a hallmark of multiple myeloma (MM), an incurable plasma cell malignancy. MM cells secrete transforming growth factor-beta (TGFβ), promoting an immunosuppressive microenvironment. We hypothesized that vactosertib, a potent, orally-available, TGFβ type I receptor kinase inhibitor, could synergize with the cereblon inhibitor, pomalidomide, to treat MM patients. We conducted a 3+3 dose-finding study of vactosertib and pomalidomide in relapsed and/or treatment-refractory MM patients who had received ≥ 2 lines of chemoimmunotherapy. Vactosertib combined with pomalidomide was well-tolerated at all dose levels and induced durable responses with 82% progression-free survival (PFS-6) at 6 months, while pomalidomide alone historically achieved only 20% PFS-6. Combined treatment with vactosertib and pomalidomide in vitro demonstrated synergistic antimyeloma activity against MM cell lines and patient tumor cells. Vactosertib reversed TGFβ-mediated expression of the immunoinhibitory receptor PD-1 on patient $CD8^+$ T-cells and improved the T-cells' autologous MM cytotoxicity ex vivo. Vactosertib reduced the TGFβ levels and the number of PD-1-expressing $CD8^+$ T-cells in patient samples while improving $CD8^+$ T-cell viability and inflammatory cytokine production. Taken together, vactosertib may help overcome immune escape, reinvigorate T-cell fitness, and improve the efficacy of immunotherapies in heavily-pretreated MM patients refractory to conventional agents.

21

Insights into heterologous immunity: ELISpot analysis of diverse mouse strains

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Enzyme-linked immunosorbent spot (ELISpot) assays are a classic technique to quantitatively measure the frequency of cytokine secretion from single antigen-reactive cells. In clinical transplantation, ELISpot assays can be used to measure the pre-transplant frequency of donor-reactive heterologous memory T cells, which correlates with graft survival and function. In murine transplant models, ELISpot assays can likewise be used pre- or post-transplant to measure the frequency of donor-reactive heterologous memory T cells. A prototypic murine solid-organ transplant model is an A/J (H-2^a) to C57BL/6 (H-2^b) transplant because of complete MHC disparities between these two strains. However, no one has done a systematic characterization of mouse strain combinations to determine which mouse strains respond strongest and weakest to allogeneic transplant. Here we systematically characterized the frequency of donor-reactive heterologous memory T cells in unsensitized mouse splenocytes. Using the ELISpot assay, we measure the frequency of donor-reactive heterologous memory CD8 T cells as a function of IFNγ and granzyme B production following 48 hours of culture of splenocytes from different mouse strains. We identify C57BL/6 mice as robustly responding to a variety of allogeneic stimuli, including the classic A/J as well as BALB/c (H-2^d). Of particular interest, although A/J splenocytes potently stimulate C57BL/6 heterologous memory T cells, A/J do not respond to C57BL/6 stimulation. This systematic review of murine responses to allogeneic stimulation can be used to guide future basic and translational transplant research by informing researchers on which mouse strain pairs have the strongest and weakest heterologous immune responses to allogeneic stimulation.

22

Large-scale functional characterization of the STIM1 cEFhand reveals residues important for calcium influx and immune response

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Calcium signaling regulates a range of biological processes, including gene transcription, muscle contraction, and cell death. Amplification of intracellular calcium signaling occurs through store operated calcium entry (SOCE), where reduced calcium in the ER causes ER transmembrane calcium “sensor” STIM1 to activate the plasma transmembrane channel ORAI1, resulting in cytoplasmic calcium influx. Coding mutations in STIM1 can dysregulate SOCE, causing immunodeficiency by reducing transcription of key immune response genes such as NFAT and NFkB. A functionally important domain of STIM1 is the ER lumenal canonical EFhand (cEFhand), responsible for sensing the depletion of ER calcium through release of a bound calcium ion. Currently, only a few missense mutations in this domain are well-characterized and it is not known how other mutations affect SOCE and immune response. We conducted a large-scale functional characterization of every missense mutation within the EFhand domain of STIM1 by ascribing functional scores based on calcium influx in cells with or without endogenous STIM1 knocked out. We found that there are certain positions within the cEFhand domain, distinct from the calcium binding motif, that are required for SOCE. There were differences in functional scores dependent on the existence of endogenous unmutated STIM1, with some variants requiring a WT STIM1 copy to exhibit a high calcium influx phenotype, including previously characterized pathogenic variants. We assessed the structural and pathogenic consequences of these variants, and debut preliminary data on validation of these scores using NFAT and NFkB transcriptional reporters to understand the effect of these variants on immune response.

23

Rapid attrition is a common feature of IgA responses within the intestine post-antibiotic treatment

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IgA responses targeting the microbiota are central to the maintenance of intestinal homeostasis. Our laboratory has recently identified a novel member of the murine microbiome, *Lachnospiriceae* A2, capable of inducing a robust CD4 T cell-dependent IgA response within the small intestine. Unlike CD4 T cell-dependent IgG responses which typically last months to years' post-induction, this IgA response wanes in less than 48 hours upon vancomycin-mediated elimination of *Lachnospriceae* A2. Similarly, treatment of Taconic mice, mice colonized with the well-characterized IgA inducing microbe segmented filamentous bacterium, with ampicillin leads to the same rapid attrition of luminal IgA. That we observe the same abrupt drop in luminal IgA in two independent systems supports the novel premise that IgA responses are exquisitely sensitive to rapid modulation of the microbiome through yet to be defined mechanisms. Elucidation of the cellular, molecular, and physiological underpinnings of these observations will inform the field's knowledge of mucosal immune responses and how antibiotics alter their dynamics.

24

O-GlcNAcylated NF-κB c-Rel is a transcriptional regulator of acute myeloid leukemia

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Acute Myeloid Leukemia (AML) is caused by myeloid progenitor cells that undergo malignant transformation which prevents differentiation and induces proliferation. NF-κB transcription factors have been shown to regulate survival, proliferation, and differentiation of AML cells. Around 40% of AML patients exhibit constitutive NF-κB activity which correlates with poor prognosis and increased resistance to chemotherapy. NF-κB proteins are post-translationally modified by O-GlcNAcylation, which control their activity. Protein O-GlcNAcylation is enhanced in AML cells and higher levels of O-GlcNAcylation causes increased NF-κB activity. We have shown that c-Rel is O-GlcNAcylated at S350 in AML and that this modification regulates c-Rel's ability to bind to specific promoter regions and control transcriptional activation. To study the role of S350 O-GlcNAcylated c-Rel and examine the potential benefit of targeting this specific modification in AML, our lab has developed a novel 4-mer peptoid called OGC350 which specifically targets and blocks the function of S350 O-GlcNAcylated c-Rel. We show that OGC350 treatment decreases viability of AML cells and suppresses expression of anti-apoptotic genes. Thus far, our data indicates that specific targeting of O-GlcNAcylated c-Rel has therapeutic promise for AML treatment by preventing activation of aberrant c-Rel activity while preserving normal functions of non-O-GlcNAcylated c-Rel.

25

VCAM1-expressing osteosarcoma cells increase metastatic potential through VCAM1-VLA4 signaling axis in macrophages

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Osteosarcoma is a highly metastatic, aggressive bone sarcoma primarily affecting children and young adults. Approximately 20% of patients present with pulmonary metastasis reducing their five-year survival rate to less than 30%. We propose that aberrant expression of Vascular Cell Adhesion Molecule 1 (VCAM1) on the tumor surface is an indicator of metastatic potential. However, the mechanism linking VCAM1 expression to metastasis is unclear. We speculate that binding of tumoral VCAM1 and its partner integrin, α4β1 (VLA4) on macrophages is pivotal in this process. Preliminary data suggest that one way the osteosarcoma tumor microenvironment (TME) shapes macrophages into a pro-tumoral "M2-like" phenotype is through the binding of VCAM1 and VLA4. This interaction can be accomplished by tumor-surface VCAM1 or by tumor-secreted soluble VCAM1 (sVCAM1). Data have shown that this interaction upregulates expression of the enzyme Arginase 1 (Arg1), a well-accepted marker of M2-polarized macrophages. Our theory that VCAM1 is directly correlated with metastatic potential is supported by the active clinical trial, NCT03811886, at University Hospitals in which the antibody targeting the α4 integrin, Natalizumab, is being used to treat pulmonary metastatic osteosarcoma. We speculate that PI3K-AKT signaling may facilitate upregulation of M2 genes. Our future studies aim to evaluate this hypothesis by assessing protein expression in bone marrow-derived macrophages (BMDMs) and alveolar macrophages influenced by osteosarcoma cell lines: K7 and K7M2, representing non-metastatic and metastatic phenotypes, respectively. Elucidating these mechanisms may lead to the development of novel cancer therapies by disrupting the VCAM1-VLA4 interaction to shift macrophage polarization towards an anti-tumoral state.

26

Identification of novel biomarkers of immune toxicity from CAR-T cell therapy using ultrasensitive NULISA™ proteome technology

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The efficacy of anti-CD19 chimeric antigen receptor (CAR) T-cell therapy in large B-cell lymphoma (LBCL) patients is limited by acute toxicities, namely cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). Previous biomarker studies have been constrained by relatively narrow protein panels and limited time points. Here, we employed NULISA, a novel ultrasensitive assay capable of simultaneously quantifying 204 proteins, to identify temporal proteome associations with acute toxicities in LBCL patients treated with anti-CD19 CAR T-cell therapy. Plasma samples from 80 LBCL patients who underwent anti-CD19 CAR T-cell therapy, collected before and after cell infusion were analyzed with NULISA. Baseline demographics and treatment toxicities including CRS and ICANS were graded according to ASTCT consensus criteria. Differential protein abundance, pathway enrichment and network analysis were performed. The strongest upregulated pathway associations of severe CRS and ICANS were inflammatory response, IL-17 signaling, non-genomic action of vitamin D3, regulation of leukocyte proliferation, and cellular extravasation. Downregulated pathways included anti-microbial humoral response and TNFs binding to receptors. In addition to previously identified inflammatory proteins, our analysis revealed significantly higher levels of Th2 cytokines, exhaustion markers, and feedback regulators, and lower levels of growth/repair factors, revealing exquisite temporal regulation of activators and inhibitors during CRS and ICANS development. This study represents the most comprehensive characterization of proteomic immune response to CAR T-cell therapy to date. Using NULISA technology, we identified novel proteins, pathways, and networks associated with CAR T-cell-induced toxicity, implicating them as potential biomarkers.

27

Mast cell-derived histamine is a negative regulator of hematopoietic stem cell function

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Hematopoietic stem cell (HSC) transplantation (HCT) is a lifesaving procedure to treat numerous hematological diseases including multiple myeloma, acute myeloid leukemia, and non-Hodgkin's lymphoma. However, the therapeutic utility of HCT is limited due to complications with graft versus host disease and infections caused by a failure to engraft neutrophils and platelets. Thus, there is a significant unmet need for novel therapeutics to improve the safety and efficacy of HCT. This project has generated compelling data demonstrating that mast cell (MC)-deficient "SASH" mice display significant increases in the phenotypic hematopoietic stem cell populations within their bone marrow (BM) and splenic compartments. Additionally, these mice demonstrate enhanced resistance to myeloablative chemotherapy as evidenced by significant survival extension compared to WT animals following weekly 5-Fluorouracil treatment. The SASH BM microenvironment also promotes accelerated engraftment and recovery following HCT of WT BM, as evidenced by increased BM cellularity and HSC populations in SASH versus WT recipients at D21 post-transplantation. Excitingly, the hematopoietic phenotype observed in SASH mice was phenocopied pharmacologically following *in vivo* administration of ketotifen, an FDA-approved antihistamine, which showed significant increases in BM cellularity and HSC populations. This work defines a previously uncharacterized role for MCs in regulating HSC function and MC targeting agents in potentially promoting hematopoietic regeneration. Moving forward we will evaluate FDA-approved antihistamines and their effect on phenotypic HSC, especially in a transplant setting. Overall, we will determine the therapeutic utility of FDA-approved antihistamines to promote hematopoietic recovery, with broader implications for HCT and additional hematopoietic malignancies.

28

Inhibiting 15-PGDH prevents blood-brain barrier deterioration and blocks neurodegeneration in Alzheimer's disease

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Even with its global prevalence and the significant economic impact it imposes, there are currently no medications available for the treatment of Alzheimer's disease (AD). While existing treatment methods focus on conventional theories related to amyloid and tau, the breakdown of the blood-brain barrier (BBB) has been identified as a crucial pathological event preceding cognitive impairments. In this study, we discovered a novel therapeutic target, 15-hydroxyprostaglandin dehydrogenase (15-PGDH)

enzyme that mediates BBB deterioration in AD. 15-PGDH enzyme degrades various eicosanoids, including prostaglandin D2 and E2 and increased 15-PGDH has been shown to be a hallmark of aging in multiple peripheral organs. Interestingly, we observed a similar increase of 15-PGDH enzymatic activity in the brains of both human and mouse with aging and AD. Therefore, we hypothesized a deteriorative role of brain 15-PGDH in AD pathology. We found 15-PGDH was primarily expressed in brain myeloid cells at the BBB and confirmed its enzymatic activity for the first time in the brain. Remarkably, both genetic and pharmacologic approaches, which inhibits 15-PGDH activity prevented both structural and functional damage of the BBB in 5xFAD mouse model of AD. Additionally, inhibition of 15-PGDH protected 5xFAD mice from oxidative stress, neurodegeneration, and cognitive impairment without altering amyloid pathology in the brain. Taken together, 15-PGDH might represent a potential therapeutic target for protecting the BBB that deteriorates in AD and other aging-related neurodegenerative diseases.

29

mtDNA release and TLR9 signaling enhance ischemia-reperfusion injury and costimulatory blockade resistant memory T cell activation in high-risk allografts

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Two important risk factors for poorer solid-organ transplant outcomes are: (1) the pre-transplant presence of donor-reactive T cells in the recipient, and (2) the length of pre-transplant graft cold ischemic storage (CIS) time. We have previously shown using a mouse heart allograft model that the sustained high-inflammatory environment generated after reperfusion of allografts subjected to prolonged (8hr) CIS is necessary for sufficient activation of donor-reactive memory T cells to mediate early costimulatory blockade resistant acute rejection. However, the mechanism by which prolonged CIS enhances early infiltration and proliferation of donor-reactive endogenous memory T cells remains poorly understood. Ischemia-reperfusion injury (IRI) is characterized by early mitochondrial dysfunction, leading to the release of mitochondrial damage associated molecular patterns (DAMPs) that can exacerbate inflammation. High risk (8hr CIS) mouse heart allograft recipients had higher levels of circulating cell-free mitochondrial DNA (cf-mtDNA) than low risk (0.5hr CIS) graft recipients during the first 24 hours post-reperfusion. Similarly, human livers that released more cf-mtDNA during pre-transplant ex vivo perfusion had poorer early clinical outcomes. We then investigated the contribution of TLR9, an innate immune sensor of cf-mtDNA, to early post-reperfusion inflammation and activity of donor-reactive memory T cells. 8hr CIS mouse heart transplants using TLR9 deficient donor allografts or recipients demonstrated reduced early proliferating graft infiltrating T cells and prolonged survival with costimulatory blockade. These findings indicate that IRI-induced cf-mtDNA release and TLR9 activation are novel targets to dampen early graft inflammation and abrogate CTLA-4Ig resistant donor-reactive memory T cell activation within high-risk allografts.

30

M2 macrophages exhibit enhanced environmental sensing of sialylated glycans via the inhibitory receptor CD22

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Glycans modulate the effector functions of immune cells through multiple families of glycan binding proteins including C-type lectins, galectins, and Siglecs. This activity is closely tied to ligand composition and receptor signaling. Siglecs (sialic acid-binding immunoglobulin-like lectins) bind sialic acid residues of glycans and typically inhibit signaling by recruiting phosphatases to proximal signaling complexes. ST6Gal1 is the sialyltransferase responsible for the addition of α2,6-linked sialic acids to terminal galactoses. In a liver-specific knockout of ST6Gal1, which lacks α2,6 sialylation on its hepatocytes, liver-resident macrophages show a pro-inflammatory skew, and the mice develop severe fatty liver disease. Siglec-2 (CD22) is unique in its restricted specificity for α2,6-linked sialic acids. Although CD22 is known as a B cell marker, we discovered that CD22 is also expressed by macrophages. Resting M0 macrophages modestly expressed CD22, but anti-inflammatory M2 macrophages robustly upregulated CD22, consistent with its ability to limit pro-inflammatory B cell signaling. Additionally, tissue-resident M2-like liver and lung macrophages showed increased CD22 expression as compared to pro-inflammatory M1-like splenic and peritoneal macrophages. WT CD22 negative macrophages also showed reduced endocytosis and processing of sialylated antigens. Since CD22 is a potent endocytic receptor, impaired endocytosis of α2,6-sialylated proteins in the absence of CD22 suggests that it removes sialylated self-molecules from the local environment, similar to the function found in wound healing-polarized macrophages. Our ongoing work focuses on determining the molecular action of CD22 in macrophages and understanding the relationship between tissue sialylation and the polarization of macrophage effector function.

31

Cervical immunity is altered in Canadian women who have bacterial vaginosis

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Bacterial vaginosis (BV), which is associated with negative reproductive health outcomes, impacts approximately 30% of women. BV is characterized by depletion of Lactobacillus and replacement with diverse anaerobic species. Treatment for BV is successful in approximately 80% of women, but over 50% symptom recurrence within 1 year, and the mechanisms responsible are not understood. We have established a prospective study of BV+ and BV- women to investigate molecular factors associated with BV treatment success. Cervical cytobrushes and cervicovaginal lavage (CVL) were collected at enrollment and months 1 and 6. Cytobrushes were immunophenotyped by flow cytometry and CVL was analyzed by label-free mass spectrometry. Clinical BV was diagnosed by Amsel criteria or Nugent scoring and treatment was provided. Molecular BV was classified as non-Lactobacillus dominant (nLD) microbiota where <50% of bacterial proteins were annotated to Lactobacillus. Fifty-two women recruited and 16 (30.8%) were diagnosed with BV.Mass spectrometry identified 5,963 bacterial proteins across 22 genera. Clinical and molecular BV classifications were consistent, with 68.8% of women diagnosed with clinical BV having nLD microbiota. Nine BV+ women responded to treatment at month 1, with three experiencing a recurrence of symptoms by month 6. BV+ women had significantly decreased levels of CD3+ T cells (p=0.0005), including CD4+ (p=0.017), CD8+ (p=0.018), and CD4-CD8- (p=0.002) subsets. Cervical T cell subsets were significantly decreased in BV+ participants at study enrollment. Studies are ongoing to further delineate the relationship between BV and cervical immunity, which may have important implications for BV treatment.

32

B cell homeostasis and autoantibody responses are tuned by a novel functional interaction between Ezrin and Myo18A

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Cytoskeletal proteins play key roles in modulating B cell development, activation, differentiation, and immunity. We reported that B cell-specific conditional deletion of Myo18A (Myo18A BKO), an unconventional myosin family protein, leads to expansion of mature B cells and plasma cells, and elevated serum immunoglobulins. Myo18A BKO mice also developed splenomegaly and autoantibodies, demonstrating that Myo18A restricts autoreactive B cell responses. Interestingly, Myosin18A physically interacts with the membrane-cytoskeletal linker protein Ezrin, and their co-localization increases upon B cell receptor stimulation. To investigate a potential functional interaction between Myo18A and Ezrin, we generated double knockout mice with simultaneous B cell-specific deletion of both Myo18A and Ezrin (M18EzDKO). M18EzDKO mice had significantly reduced antibody-secreting cells, serum IgM levels and autoantibody production compared to Myo18A BKO. The phosphorylation and localization of Ezrin was not altered in Myo18A single KO B cells, nor was the expression and localization of Myo18A in Ezrin single KO B cells. However, bulk RNA sequencing of naïve Myo18A and Ezrin single KO B cells revealed reciprocal enrichment of specific biological pathways, including type I and II interferon response, Myc targets, estrogen response and heme metabolism. Our data suggest that Myo18A and Ezrin play opposite roles in regulating the cellular processes that enforce B cell homeostasis and differentiation. Taken together, our data reveal a novel functional interaction between Myo18A and Ezrin that tunes B cell function.

33

Mechanisms of immunomodulation of blueberries in intestinal inflammation and injury

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Inflammatory Bowel Diseases (IBDs) are complex diseases characterized by chronic inflammation of the gastrointestinal tract. Despite the diverse treatments available today, many patients are refractory to therapy or experience relapse within a year. Dietary intervention for IBD has become of interest in recent years with studies suggesting a correlation between blueberries and reduced intestinal inflammation. However, the mechanisms underlying this observation have yet to be elucidated. Using immortalized intestinal epithelial cells (iIECs) stimulated with lipopolysaccharide (LPS) *in vitro* in parallel with blueberry extract (BBE) treatment, we found that IL-6 secretion is reduced in stimulated cells treated with BBE compared to vehicle-treated controls. Our data indicates that BBE inhibits secretion of inflammatory mediators, thereby suggesting a role of BBE molecules in blocking inflammatory signaling pathways. Using ELISA, we found that BBE treatment in wild-type C57BL/6 mice with DSS-induced colitis reduced IL-6 and fecal lipocalin levels. Furthermore, we observed wound-associated epithelial (WAE) cell coverage and crypt regeneration in BBE-treated mice compared to vehicle-treated controls. Our data suggests that BBE promotes wound repair in an *in vivo* model of intestinal injury to reverse intestinal damage. Altogether, we find that BBE exhibits reproducible anti-inflammatory effects *in vitro* and pro-reparative effects *in vivo*, thereby suggesting important implications for the development of novel, effective and safe therapies for IBD.

#34

A nanobody-based complement inhibitor targeting complement Component 2 (C2) reduces hemolysis in a complement humanized mouse model of autoimmune hemolytic anemia

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C2 is an attractive therapeutic target for many complement-mediated diseases. We developed Nab1B10, a new anti-C2 nanobody that potently and selectively inhibits both the classical and lectin pathways of complement activation. Mechanistically, Nab1B10 binds to the C2 a portion of C2 and inhibits the assembly of C3 convertase C4b2a. Nab1B10 cross-reacts with monkey but not rodent C2 and inhibits classical pathway-mediated hemolysis. Using a new complement humanized mouse model of autoimmune hemolytic anemia (AIHA), we demonstrated that Nab1B10 abolished classical pathway complement activation-mediated hemolysis *in vivo*. We also developed C2-neutralizing bi- and tetra-valent antibodies based on Nab1B10 and found these antibodies significantly more potent than the other anti-C2 monoclonal antibody that is already in clinical trials. These data suggest that these novel C2-neutralizing nanobodies could be further developed as new therapeutics for many complement-mediated diseases, in which pathogenesis is dependent on the classical and/or lectin pathway of complement activation.

35

Novel inflammatory mechanism in psoriasis pathogenesis: NF-kB c-Rel regulates TLR7 signaling in dendritic cells

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Psoriasis is a chronic inflammatory skin disease characterized by epidermal hyperplasia, excessive inflammatory cytokine production, and dermal immune cell infiltration. Nuclear factor kappaB (NF-κB) c-Rel is a psoriasis susceptibility locus, however mechanisms underlying c-Rel transactivation during disease is poorly understood. c-Rel is a critical regulator of dendritic cell (DC) function and Toll-like Receptor Signaling (TLR) signaling, both of which significantly contribute to psoriasis pathogenesis. Here, we studied the previously unknown mechanism of TLR7-induced c-Rel-mediated inflammation in DCs. We found that c-Rel is highly expressed in the lesional skin of psoriasis patient transcriptomes and imiquimod (IMQ)-induced psoriatic lesions of mice. c-Rel deficiency significantly protected mice from IMQ-induced psoriasis. We found that c-Rel deficiency specifically compromised TLR7-, and not TLR9- or TLR3-, induced inflammation in dendritic cells. Mechanistically, c-Rel deficiency disrupted activating NF-κB dimers and allowed binding of inhibitory NF-κB homodimers to the IL-1β and IL-6 promoters to inhibit their expression. This functionally compromises the ability of c-Rel deficient DCs to induce Th17 polarization, which is critical in psoriasis. Our findings reveal that c-Rel is a key regulator of TLR7-mediated dendritic cell-dependent inflammation, and targeting c-Rel-dependent signaling could prove an effective strategy to dampen excessive inflammation that occurs in TLR7-related skin inflammation such as psoriasis.

36

Regulation of IgG sialylation by cytokine mediators

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The IgG antibody is a key component of the immune system, with a significant role in the regulation of the inflammatory response and downstream effects. It has been previously shown that the lack of a terminal sialic acid on the conserved glycan that lies within the Fc region of the IgG antibody is strongly correlated with an “inflammatory” state. For example, individuals with conditions such as rheumatoid arthritis, HIV, and tuberculosis typically present with decreased IgG sialylation, which promotes inflammatory responses downstream of Fc receptors on immune cells. Additionally, we have shown that the sialylation of IgG occurs via the FcRn-mediated recycling pathway in endothelial cells. Despite the established correlation between IgG sialylation and inflammatory state, the regulation of this mechanism remains unclear. Here, we seek to better understand the regulation of IgG sialylation by signaling mediators, namely cytokines such as IFNg and TNFa. Gaining insight into the regulation of IgG sialylation by cytokine mediators advances our understanding of inflammatory state modulation and furthers our progress toward developing personalized therapeutic treatments for inflammatory diseases.

37

Spatial transcriptomics reveal the Achilles heel of glomeruli in antibody mediated rejection

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Digital Spatial Profiling (DSP) resolves transcript analysis to localized structures in tissue. Using GeoMx technology, we have found that cell infiltrates and tissue injury are heterogeneously distributed. A series of 4 biopsies were available from a patient with acute antibody-mediated rection (AAMR). A baseline biopsy from 3 weeks after transplantation. This was followed by a 3–6-month biopsy that was diagnosed as acute antibody mediated rejection (AMR) (diffuse C4d+ in the context of de novo DSA to DR51, DR53 and DQ2). The patient was treated with plasmapheresis and bortezomib, a follow up biopsy five months later revealed ongoing AMR and a mild increase in tubulointerstitial fibrosis compared to the first biopsy. A fourth biopsy six months later revealed that the rejection was beginning to resolve. These serial sections were stained with CD31 and CD68 for selection of Regions of Interest (ROI's) that were drawn around whole glomeruli. A total of 19 glomeruli were collected and analyzed using the GeoMx software. Using the baseline biopsy as a control for the 3 subsequent biopsy, we found 17 genes increased and 39 decreased by 2-fold and $p<0.01$. The GeoMx deconvolution program identified increased signatures for NK cells as well as monocytes and macrophages that correlated with the increased numbers of CD68 positive cells imaged in the corresponding ROIs. HLA-DRB1 was among the 17 transcripts upregulated in the glomeruli during rejection. More detailed analysis revealed that other HLA transcripts (B2M, HLA-B, DRA, DRB1, DPA1 and DPB1) were increased above baseline in the first biopsy demonstrating AAMR.The Human Cell Atlas classified the 39 genes that were decreased during the initial rejection as characteristic of podocytes, with many being signature genes for podocytes, such as NPHS1 (nephrin) and NPHS2 (podocin). Expression of these genes did not recover in the subsequent 2 biopsies. Additional ROIs encompassing areas of tubulointerstitial fibrosis we redefined and segmented using a marker antibody for alpha smooth muscle actin (aSMA). Successful segmenting was confirmed by the high expression of ACTA2 (SMA gene) and 3 additional genes characteristic of myofibroblasts and fibroblasts (TAGLN, NDUFA4L2 and NOTCH3). Deconvoluting the cell content of the segments disclosed signatures for memory B cells in the initial AAMR sample. Treatment with plasmapheresis and bortezomib did not eliminate the signal for memory B cells in the subsequent biopsy. Macrophages and memory CD8 T cell signatures were also increased in the fibrotic areas. Collectively these data demonstrate a compartmentalization of injury processes. Innate immune cells including NK cells, monocytes and macrophages are located in glomeruli and tubule-interstitial compartments. However, adaptive immune cells including memory B and CD8 T cells localized to the tubule-interstitial compartment amid myofibroblasts. Furthermore, podocyte transcripts were decreased in glomeruli indicating a vulnerability of these cells to AAMR.

38

Nuclear estrogen receptors modulate T cell pathogenicity in an *in vivo* colitis model

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17β-estradiol (estrogen, “E2”) signals through two nuclear receptors, ERα and ERβ, to regulate gene expression in target cells, including immune cells. We previously showed that expression of ERβ was reduced in CD4+ T cells of Crohn’s disease patients, and deletion of ERβ in a mouse model of Crohn’s-like ileitis worsened inflammation selectively in females. These data led us to hypothesize that ERα-specific signaling (in the absence of ERβ) is proinflammatory in T cells, potentially in a sex-specific manner. To test this, we used the CD45RBhigh T cell transfer model of colitis to determine the inflammatory potential of T cells lacking expression of ERβ (ERβ-KO). In agreement with our hypothesis, transfer of male ERβ-KO T cells to sex-matched Rag2-/- recipients exacerbated colitis compared to transfer of wild-type (WT) cells. Unexpectedly, transfer of male ERα-KO T cells also exacerbated colitis to a similar degree, suggesting a functional redundancy for these receptors in males. Despite worsened disease in male recipients of ERα-KO and ERβ-KO T cells, there was no difference in the frequency or polarization of CD4+ T cells recovered from the spleen, mesenteric lymph node, or colon of the Rag2-/- recipients. Further interrogation of ERα-KO and ERβ-KO CD45RBhigh T cells prior to transfer showed an increased capacity for proliferation and TCR signaling via increased expression of CD45RB when compared to WT cells. Future studies will determine the mechanisms through which ERα v. ERb mediate CD4+ T cell proliferation and activation, and how this contributes to increased T cell pathogenicity and worsened colitis.

39

Assessing antibody-bound microbes in the female reproductive tract

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The vaginal microbiome (VM) plays a key role in women's health outcomes. A *Lactobacillus* dominant microbiome, specifically *L. crispatus*, is associated with reduced pH and increased expression of epithelial integrity-related proteins while non-*Lactobacillus* dominant microbiome often characterizes vaginal dysbiosis. The balance of VM microbiota likely depends on both bacterial and host immune factors. Recent evidence shows that women with *L. crispatus*-dominated VMs have increased IgA antibody coating of bacterial cells, suggesting that host antibody binding to vaginal microbiota may play a role in vaginal health and disease. However, the role of antibody bound and unbound microbes in the VM during states of vaginal dysbiosis like cervical dysplasia is underexplored. We hypothesize that women with vaginal dysbiosis will have a decreased proportion and abundance, as well as altered functional profiles, of antibody-bound bacteria compared to women with normal tissue. To test this, we developed a method to assess the percentage of IgA+, IgG+ and IgA+IgG+ bacteria in vaginal swabs. Additionally, we optimized magnetic activated cell sorting to isolate IgA-bound and unbound bacteria for 16S rRNA analysis. Our preliminary data demonstrate that we can detect IgA+, IgG+, and IgA+IgG+ bacteria in vaginal swabs and observe differences in the abundance of various bacterial taxa bound or unbound to IgA. Future work includes testing vaginal swabs from an observational cohort of women with cervical cancer to determine whether the abundance and functions of IgA+, IgG+ and IgA+IgG+ bacterial taxa differ in women with or without cervical neoplasia. This work will further our understanding of VM health vs dysbiosis, and their contributions to women's health.

40

Specific Macrophage States and Select *Debaryomyces hansenii* Strains Promote Immune Evasion

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D. hansenii is a constituent of the gut microbiome due to its prevalence as a food microbe. Our lab identified culturable *D. hansenii* within the ulcers of a subset of Crohn Disease patients. In mouse models of intestinal injury, *D. hansenii* persisted within macrophages and hindered repair. Our hypothesis is that *D. hansenii* persists within macrophages because of the immune evasion capacity of this microbe that can be modulated by host factors such as expression of innate immune receptors on the macrophage. I tested candidate pattern recognition receptors for their ability to mediate phagocytosis and induce TNF from the macrophage line RAW264.7. I observed that Dectin1 neutralization resulted in a 50% reduction in phagocytosis and a 70% reduction in TNF secretion. I validated these results using primary Bone Marrow Dendritic Cells (BMDCs) and Bone Marrow Macrophages (BMDMs) and observed that Dectin1 is required for phagocytosis by both lines, but interestingly, only BMDCs can secrete TNF following infection. These data support my hypothesis that myeloid cells have different responses to *D. hansenii*. This may mirror *D. hansenii* persistence within macrophages *in vivo*. I next tested the hypothesis that strains of *D. hansenii* may evade Dectin1 phagocytosis. I tested Dectin1 mediated phagocytosis of three food-isolates and two patient-isolates of *D. hansenii*. Two food-isolates were able to evade Dectin1, while both patient isolates and one food isolate was phagocytosed in a Dectin1-dependent manner. Our findings suggest that immune evasion by *D. hansenii* is context dependent with both microbial and host phagocyte phenotypic effects.

41

TME-gated inducible CAR (TME-iCAR) T-cell therapy for solid tumors

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Autonomous “living drug” Chimeric Antigen Receptor (CAR)-T cell therapy has revolutionized cancer medicine. Despite promising clinical results, these engineered T cells exhibit significant challenges due to overactivation, and lack of tumor-specific antigens which can cause severe on-target off-tumor toxicity. Developing regulatory systems to provide spatial and temporal control over CAR-T cell activities is needed. Here, we present a novel strategy using a resembled genetic “AND” gate that integrates chemically-induced proximity (CIP) and cancer-activatable prodrug strategy to generate the next generation of CAR-T cells (TME-iCAR T-cell) that are capable of sensing multiple tumor-specific characteristics (e.g., tumor antigens and tumor microenvironment (TME) signals) to precisely execute therapeutic functions within the TME. This new technology was built on the abscisic acid (ABA) -based CIP and its associated reactivity-based caging/sensing technology. Two hypoxia-responsive small molecule prodrugs were designed by conjugating ABA with different nitroaromatic derivatives. We tested TME-iCAR T cells specific for two antigens in vitro and found remarkable cancer signals-restricted activation and cytotoxicity to cancer cells, while their maximal activities were similar to second-generation (2G) CAR-T cells. We also showed their controllability in vivo using a xenograft hypoxic prostate tumor model. This design allowed for reversible and “tunable” control of CAR-T cell activity *in vitro* and *in vivo*. Our highly modular multi-signal requirement control system represents a new promising strategy to enhance the safety of CAR-T cell therapies.

#42

Cholesterol biosynthesis pathways in monocyte-derived macrophages link cytomegalovirus infection to atherosclerosis

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Cytomegalovirus (CMV), a widespread herpesvirus that establishes latent infection in hematopoietic progenitor cells, is associated with increased inflammation and inflammation-related disorders, particularly cardiovascular disease (CVD). CMV can reactivate as latently-infected cells differentiate into macrophages. Reactivation is usually asymptomatic in immunocompetent hosts but may contribute to CMV-associated inflammation and CVD. To examine these macrophages more closely, RNA sequencing was performed on monocyte-derived macrophages (MDMs) generated using a model in which circulating monocytes were differentiated with autologous serum, preserving the influence of systemic biomarkers found *in vivo*. Differential gene expression analysis identified over 700 genes that were significantly upregulated in MDMs derived from CMV-seropositive donors (nominal $p<0.05$), with an enrichment in lipid and cholesterol synthesis pathways. Using the TRRUST database, SREBP1 and SREBP2, known regulators of lipid metabolism, were identified as significant drivers of expression of upregulated genes, including *FDFT1*, *FDPS*, and the statin target *HMGCR*. In a separate dataset, *FDFT1* expression was associated with viral load in *in vitro* CMV infection, suggesting that MDMs from people with CMV are transcriptionally permissive for CMV replication. A gene set of the top 100 most significantly upregulated genes in MDMs from people with CMV was enriched in bulk tissue from atherosclerotic plaques compared to peripheral blood cells and among macrophages isolated from ruptured atherosclerotic plaques compared to those from stable plaques, consistent with a proatherogenic role for MDMs in people with CMV. Taken together, our findings link cholesterol biosynthesis pathways in MDMs to CMV infection and atherosclerosis.

43

Inflammasome activation drives parallel signaling pathway inactivation

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Inflammasomes trigger in response to both endogenous and exogenous danger signals (DAMPs and PAMPs.) The resulting pyroptotic cell death drives both the release of cytokines and the recruitment of immune cells. While the pathways that lead to inflammasome assembly and gasdermin cleavage have been well studied, little is known about the effect of inflammasome activation on concurrent signaling pathways and these pathways' effects on pyroptosis-independent gene expression. Pan-phosphoproteomics identified previously unknown pathways affected by NLRP3 inflammasome activation. Western blot and cell death assays were coupled with RNA seq analysis to determine inflammasome-directed signaling effects on gene expression. Rapid ERK1/2 inactivation occurred within minutes of inflammasome stimulation. Remarkably, most other signaling pathways were unaffected, and this effect was independent of cell death. This ERK inactivation had a global effect on the cell, as RNA seq analysis revealed that rather than a global loss of RNA, subsets of expressed mRNA, including cytokine- and chemokine-encoding genes, were resistant to degradation. Additionally, biochemical analysis suggests that ERK inactivation primes the cell to undergo apoptosis should pyroptosis fail. ERK activity is specifically downregulated in response to inflammasome activation. This has widespread cell biological consequences, including effects on mRNA stability and apoptotic priming, setting the stage for an alternative death method in the face of failed pyroptosis.

44

The role of macrophage Piezo1 on bacterial clearance in cystic fibrosis

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Cystic Fibrosis (CF) is a multisystem disease caused by mutations in the Cystic Fibrosis Transmembrane Regulator (CFTR) gene resulting in chronic pneumonia. Pseudomonas *aeruginosa* is a common pathogen in CF which causes lung injury through its virulence factors, such as flagellin. In addition to soluble bacterial signals, macrophages require lung matrix signals through mechanosensitive ion channels, such as Piezo1, for effective bacterial clearance through phagocytosis. CF lung macrophages have impaired phagocytosis, but the cause is poorly understood. To determine the role of macrophage Piezo1 on phagocytosis in CF, we measured Piezo1 Ca^{2+} channel function (FLIPR 5 Ca^{2+} Assay, Molecular Devices), mRNA, and total protein which were reduced in CFTR-mutant (R117H, impaired Cl^- conductance) compared to wild-type (WT) bone marrow-derived macrophages (BMDMs). Next, we measured phagolysosome maturation, a key step in phagocytosis, ± *P. aeruginosa* flagellin (Invivogen) using pHrodo Zymosan particles (Invitrogen). Flagellin-induced phagolysosome maturation was reduced in the CFTR R117H BMDMs by 46.6%. Phagolysosome maturation was restored in CFTR R117H BMDMs upon activation of Piezo1 by its agonist, Yoda1. *In vivo*, inhibition of Piezo1 decreased bacterial clearance and increased inflammatory cell infiltration in the lungs in a chronic *P. aeruginosa* pneumonia mouse model. Piezo1 mRNA was reduced in human CF peripheral blood mononuclear cells and Piezo1 activation increased phagolysosome maturation in healthy human alveolar macrophages by 63.8%. Taken together, our data demonstrate that macrophage Piezo1 is reduced in CF and activation of Piezo1 restores phagocytosis in CFTR-mutant macrophages. Therefore, Piezo1 is a possible therapeutic target against CF chronic pneumonia.

45

A novel protective role for Hmgb1 in the gastrointestinal lumen

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High mobility group box-1 (HMGB1) is a ubiquitously expressed protein with a variety of functions depending on its location and post-translational modifications. Previous data has shown that HMGB1 levels are increased in the stool of patients with inflammatory bowel disease (IBD), yet in the mucosa it is reduced. We set out to understand the role of HMGB1 in the gastrointestinal (GI) tract lumen in IBD. We utilized mice conditionally deficient in intestinal epithelial cell HMGB1 (DIEC). Fixed colon sections stained with anti-HMGB1 antibody revealed a thick ribbon of HMGB1 present at the apical surface of the IECs. HMGB1 appeared to be bound to bacteria present in the lumen, confirmed via flow cytometric analysis. We tested the functionality of HMGB1 two ways and concluded that *E. coli* more easily infiltrated mucus from DIEC mice and that HMGB1 causes the bacteria to aggregate. Immunofluorescence (IF) for FimH in the DIEC mice revealed increased protein in the absence of HMGB1. Stained colons from ulcerative colitis (UC) and non-IBD patients revealed UC patients had an increase in FimH but a reduction in HMGB1 expression compared to non-IBD controls. Extracellular HMGB1 has been linked to cell damage and inflammation in the gut during IBD. However, our studies have identified a new and protective role for HMGB1 in the gut lumen that is compromised during IBD. In this role, HMGB1 functions as a protective barrier against the resident microbiota and appears to promote a commensal state in resident gut microbiota.

46

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

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Necroptosis is required to maintain tissue homeostasis. While this form of cell death clears pathogens and cancer cells, when dysregulated, it can also cause devastating tissue damage especially in ischemia-reperfusion and chemical-induced injuries. A detailed biochemical mechanism of necroptotic execution is necessary to identify the best targets for pharmaceutical intervention to prevent necroptotic tissue damage. Biochemically, necroptosis is tightly regulated by multiple phosphorylation events, leading to the final phosphorylation of the executioner protein MLKL by RIPK3. The exact purpose of this phosphorylation event is not well-defined but assumed to promote MLKL oligomerization and translocation to internal membranes, leading to cellular demise. However, this phosphorylation event alone is not sufficient to cause cell death. This is best demonstrated by the fact that phosphomimetic MLKL cannot cause cell death, as well as the observation that certain MLKL mutants are phosphorylated in the absence of cell death. These results suggest that, in addition to RIPK3-mediated phosphorylation, there are additional requirements for MLKL activation and subsequent necroptotic cell death. To elucidate these additional requirements, we performed mutational analysis of MLKL and investigated its effect on MLKL phosphorylation, oligomerization, and cell death. Surprisingly, we observed that mutations in the MLKL N-terminus were able to prevent cell death but not phosphorylation of C-terminus. Our results are consistent with a model where the N-terminal executioner domain of MLKL is released from the brace prior to phosphorylation by RIPK3. We further tested this model using pharmacologic inhibitors and neutralizing monobodies against MLKL.

47

Development of cellular assays to characterize the impact of sequence variation on STING function in bats

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Bats are reservoirs for many deadly human viruses. Genomic and experimental evidence suggests that bats have evolved a balanced immune response that controls infection while minimizing systemic inflammation. Minimization of inflammation likely evolved to reduce pathogenesis from cellular damage caused by metabolically intensive independently powered flight. The cGAS-STING pathway is important for pathogen detection by sensing cytosolic DNA from infection or cellular stress and triggers an immune response by upregulating cytokines, IFNs, and autophagy. STING was found to have a dampened IFNb response in bats due to a phosphosite mutation. However, reversion of the mutation did not relieve reduced signaling in some of the bat species tested. We hypothesize that diverse bat STING (bSTING) orthologs will have a range of molecular mechanisms that downregulate immune signaling and upregulate autophagy compared to human STING. Furthermore, we predict that bat STING will be better at resisting inhibition by viral immune evasion proteins. We are developing three high throughput single cell assays to test the functionality of diverse bSTING orthologs. The first assay measures STING activity based on cell survival, with results that show when activated overexpressed human STING cells have ~90% death rate when compared to no treatment. The second assay utilizes an IRF3 promoter driven GFP reporter, wherein STING activation increases GFP positivity 14-fold. The third assay measures autophagosome formation through LC3b puncta generated upon STING induction. Once the reporter systems are optimized, a panel of 51 bSTING orthologs will be screened to determine their activity compared to human STING.

#48

Immunoproteasome Activation Expands The MHC Class I Antigenic Landscape, Unmasks Neoantigens And Reduces Tumor Growth

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The majority of T-cell responses involve proteasome-dependent protein degradation and the downstream presentation of antigenic peptides complexed with major histocompatibility complex (MHC) class I (MHC-I) molecules that are presented to $CD8^+$ cytotoxic T-cells. However, immune evasion is a cancer hallmark that is achieved by disruption of host antigen processing and presentation machinery. Consequently, mechanisms of immune evasion promote cancer growth and survival as well as the acquisition of de novo and acquired resistance to immunotherapy. Immunoproteasomes represent a highly specialized proteasomal variant that degrades proteins in cells exposed to oxidative stress and proinflammatory stimuli. We hypothesized that immunoproteasome activators would increase neoantigen presentation and promote T-cell activity. We performed a high-throughput screen identifying a novel molecular entity (Compound A) that specifically increased immunoproteasome activity and subsequently expanded the multiple myeloma (MM) patient immunopeptidome. Compound A increased presentation of individual MHC-I-bound peptides >100-fold and unmasked tumor-specific neoantigens on myeloma cells. Global proteomic integral stability assays determined that Compound A binds the proteasome structural subunit PSMA1 and promotes association of the proteasome activator PA28a/ (encoded by PSME1/PSME2) with immunoproteasomes. Genetic silencing of PSMA1, PSME1, or PSME2 as well as treatment with immunoproteasome-specific suicide inhibitors abolished the effects of Compound A on antigen presentation. Treatment of MM cell lines and patient bone marrow $CD138^+$ cells with Compound A increased the anti-myeloma activity of both allogenic and autologous cytotoxic T-cells. Taken together, our results demonstrate the paradigm-shifting impact of immunoproteasome activators to expand the myeloma immunopeptidome and reveal actionable neoantigens for personalized T-cell-directed immunotherapy.

49

The mechanism of plasma membrane rupture at the end of pyroptosis and necroptosis

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NINJ1 is a recently identified active executioner of plasma membrane rupture (PMR), a process previously thought to be a passive osmotic lysis event in lytic cell death. NINJ2 is a close paralog of NINJ1 but unable to mediate PMR. By cryoEM, we find that both NINJ1 and NINJ2 assemble into a linear filament with their N-terminal amphipathic helix crosslinking neighboring subunits. One side of the filament is hydrophobic and strongly associates with lipids, while the other side is hydrophilic and water-soluble. We propose a mechanism that NINJ1 mediates PMR mainly by wrapping around membrane fragments and solubilizing them like lipid nanodiscs, and in a less extent, forming ring-like structures and making large pores on the plasma membrane. In this sense, NINJ2's incapability of mediating PMR is attributable to its intrinsic curvature that prevents the formation of nanodisc-like structure or a closed ring. Moreover, the curvature of NINJ2 is likely induced by strongly associated lipids, particularly a cholesterol, in the inner leaflet of the lipid bilayer, which function like molecular glues to stick together the lower half of each NINJ2 subunit.

50

Skin microbiota drive checkpoint inhibitor toxicity in a genetic autoimmune susceptible mouse

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The clinical success of immune checkpoint inhibitor (ICI) therapy with anti-CTLA-4 and anti-PD-1 is limited by increased incidence of immune-related adverse events (irAEs), presenting a growing challenge for patient care; patients with pre-existing autoimmune disease are at greatest risk of developing them. Preclinical models of irAEs are difficult since WT mice are not susceptible to ICI toxicity. We report a model wherein genetic autoimmune susceptibility coupled with environmental drivers lead to aberrant B cell activation, which has been underappreciated in the context of irAE development. ICI treatment of young *Act1*$^{-/-}$ mice expedited and exacerbated autoimmunity. With 5 weeks of treatment, ICI *Act1*$^{-/-}$ developed a failure to thrive phenotype with attenuated weight gain and periocular skin lesions in comparison to IgG *Act1*$^{-/-}$, IgG *Act1*$^{+/-}$, and ICI *Act1*$^{+/-}$ controls. The microbiome is a critical synthesizer of the environment and a known modulator of ICI efficacy, suggesting that intestinal microbiota composition may predispose individuals to irAEs. Consistent with this, GF *Act1*$^{-/-}$ mice do not develop irAEs, but co-housing GF and SPF *Act1*$^{-/-}$ mice was permissive for ICI toxicity. However, colonization with intestinal microbes from multiple different sources failed to render GF *Act1*$^{-/-}$ mice susceptible. This led us to consider that another source of microbes was required. Total skin microbiota transfer from SPF *Act1*$^{-/-}$ mice rendered GF mice susceptible to ICI toxicity. 16S rRNA and *dnaJ* sequencing showed that skin microbiota transfer was permissive for the inoculation of *Staphylococcus epidermidis* and *xylosus*. These efforts highlight the potential of de-coupling a modifiable microbiome with genetic autoimmune susceptibility to attenuate irAEs.

51

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

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), is a bacterial pathogen that 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. Our lab recently published that M1, but *not M2-like* macrophages infected with Mtb can activate Mtb-specific CD4+ T-cells. However, the mechanisms of subversion of the T-cell response remain unknown. In this study, we exposed M1 and M2 macrophages to either virulent Mtb, γ-irradiated Mtb, or Mtb whole cell lysate and performed RNAseq on the samples. Since M2 macrophages treated with either Mtb lysate or irradiated Mtb activated CD4+ T-cells, these conditions served as important controls. We curated a list of genes unique to Mtb-infected M2 macrophages that represent candidate inhibitory pathways of T-cell activation. Pathway enrichment, gene ontology, and STRING analysis were used to identify master regulatory genes, including *PROS1, GAS6,* and *P2RX4*. Once validated, expression of these targets will be knocked down in Mtb-infected M2 macrophages using RNAi, after which CD4+ T cell activation responses will be assessed using autologous Mtb antigen-specific T cell lines by flow cytometry. Our data will identify key genes responsible for the subversion of CD4+ T cell activation by Mtb-infected M2 macrophages. Importantly, our data will also expose key interactions facilitating CD4+ T cell recognition of infected macrophages that phenotypically resemble lung resident alveolar macrophages, the niche cell for Mtb infection.

52

Single cell and bulk RNA expression analyses identify enhanced hexosamine biosynthetic pathway and O-GlcNAcylation in acute myeloid leukemia blasts and stem cells

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Acute myeloid leukemia (AML) is the most common acute leukemia in adults with an overall poor prognosis and high relapse rate. Multiple factors including genetic abnormalities, differentiation defects and altered cellular metabolism contribute to AML development and progression. Though the roles of oxidative phosphorylation and glycolysis are defined in AML, the role of the hexosamine biosynthetic pathway (HBP), which regulates the O-GlcNAcylation of cytoplasmic and nuclear proteins, remains poorly defined. We studied the expression of the key enzymes involved in the HBP in AML blasts and stem cells at the single-cell and bulk level. We found higher expression levels of the key enzymes in the HBP in AML as compared to healthy donors in whole blood. We also observed elevated O-GlcNAc Transferase (OGT) and O-GlcNAcase (OGA) expression in AML stem and bulk cells as compared to normal hematopoietic stem and progenitor cells (HSPCs). Gene set analysis showed substantial enrichment of the NF-κB pathway in AML cells expressing high OGT levels. We found by flow cytometry AML bulk cells and stem cells show enhanced OGT protein expression and global O-GlcNAcylation as compared to normal HSPCs, validating our in-silico findings. Our study suggests the HBP may prove a potential target, alone or in combination with other therapeutic approaches, to impact both AML blasts and stem cells. Moreover, as insufficient targeting of AML stem cells by traditional chemotherapy is thought to lead to relapse, blocking HBP and O-GlcNAcylation in AML stem cells may represent a novel promising target to control relapse.

53

Epitope mapping of the α-FXIIa allosteric inhibitor monoclonal antibody 5C12

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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. 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. We postulate that 5C12 binding to FXIIa causes a change in the orientation of the active site histidine, incapacitating access to the catalytic triad. Use of anti-FXIIa Mabs may reduce or eliminate the need for heparin to prevent thrombosis with medical device use. Presently, there is no agent like this approved for this therapeutic indication.

54

Oral resident memory T cell immunosurveillance of taste buds

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Tissue resident memory CD8+ T cells (TRM) accelerate pathogen detection and clearance at regions of the body that interface with the outside world. The oral mucosa represents one such frontline location, yet compared to other mucosal tissues, the fate and function of TRM within the mouth has been largely unexplored. We developed a noel **V**iral-**P**rime, **E**pitope-**P**ull (**VPEP**) strategy for generating abundant and broadly distributed TRM of a defined antigen specificity in the mouths of SPF mice. Oral TRM were commonly observed within and surrounding taste buds and papillae where their local reactivation induced discernable inflammation. Utilizing bulk RNA sequencing on various taste bud enriched tissues, we asked whether peptide reactivation of oral TRM would provoke transcriptional changes in genes related to taste. Mice harboring reactivated oral TRM showed a 2- to 6-fold increase in ~33% of mouse bitter receptor genes (Tas2R's), potentially increasing sensitivity to tens-of-thousands of bitter molecules. Olfactory receptor expression in the oral mucosa was also invoked. Ongoing work is testing the hypothesis that local TRM-driven antiviral responses in the oral mucosa enhances sensitivity to bitter dietary compounds, a mechanism which may have evolved to protect the host against the accidental ingestion of harmful foods when sick.

55

CD57+ T cell transmigration through vascular endothelial cells is enhanced by TNF: A novel model of cardiovascular risk in people with HIV

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Cardiovascular diseases are the leading cause of mortality worldwide. People living with human immunodeficiency virus (PWH), despite achieving viral suppression and immune reconstitution through antiretroviral therapy, face increased risk and earlier onset of atherosclerosis than the general population. Nearly all PWH harbor latent cytomegalovirus (CMV) infection, which expands CD4 and CD8 T cells that express high levels of CD57 and the vascular-homing chemokine receptor CX3CR1. CD57+ T cells are readily recovered from atherosclerotic plaques, and our previous work has shown that the chemoattraction and cytotoxic activity of these cells are enhanced by IL-15, tumor necrosis factor (TNF), and CD2/LFA-3 co-stimulation – factors that are elevated in latent CMV infection and/or in atherosclerosis. However, the specific mechanisms facilitating the infiltration of CD57+ T cells into plaques remain elusive. Here, we report the development of a novel assay to quantify T cell adhesion to and transmigration through a primary human vascular endothelial cell monolayer. Using this assay, we show that a higher proportion of CD57+ T cells adhere to and migrate through the monolayer than CD57- cells. Moreover, activating the endothelium with TNF, which has previously been shown to elicit IL-15, CX3CL1, and LFA-3 expression from endothelial cells, significantly increases transmigration of CD57+ T cells, aligning with our earlier findings that identify TNF as a critical proinflammatory factor promoting the vascular homing of CD57+ CD4 and CD8 T cells. This model will allow us to elucidate the mechanisms of, and test interventions to prevent, CD57+ T cell infiltration into plaques.

56

Human CD4+ T cells recognize autologous bystander non-infected macrophages exposed to soluble Mycobacterium tuberculosis antigens but lack IFNγ production

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Mycobacterium tuberculosis (Mtb) infects macrophages and CD4+ T cells are critical to control infection. Studies in the mouse model suggest that only a portion of activated Mtb-specific CD4+ T cells in the lungs of infected mice recognize Mtb-infected macrophages. We tested the hypothesis that Mtb-specific CD4+ T cells recognize bystander non-infected macrophages exposed to infected macrophages using primary human cells from healthy individuals with latent Mtb infection (LTBI) and reporter virulent strain of Mtb. We found that, after sorting bystander non-infected macrophages from Mtb-infected cells, a subset of CD4+ T cells was activated in response to bystander non-infected macrophages in a TCR-dependent manner. Our findings were validated using transwell plates. Interestingly, a reduced proportion of those activated cells produced IFNγ in response to non-infected macrophages using that system. We found that M1-like macrophages shed exosomes during infection, which could hypothetically carry Mtb antigens to bystander cells. We tested the capacity of non-infected macrophages treated with either exosomes or exosome (EV)-depleted supernatants from infected M1 macrophages to activate CD4+ T cells. Only EV-depleted supernatant-treated non-infected macrophages elicited significant CD4+ T cell activation, suggesting that bystander non-infected macrophages promote CD4+ T cell activation after taking up soluble Mtb antigens from neighboring Mtb-infected cells. These findings shed light on the impact of bystander macrophages on T cell responses in tuberculosis.

57

Development of vaccines with adjuvant in patients with metabolic diseases

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With over 70% of Americans overweight including 40% with obesity, the impact of metabolic disease on immunity has reached a critical point, adversely affecting vaccine responses, autoimmunity, and infectious disease. There is strong evidence indicating individuals with metabolic diseases such as obesity do not have appropriate humoral immune responses. In addition, immune responses to vaccines wane faster in those with severe obesity than in those with a healthy body mass index (BMI). Humoral immunity relies on production of short- and long-lived plasma cells (PCs) as well as memory B cells and the proper balance of these depends on B cell-intrinsic metabolism. We hypothesize that increased basal metabolic activity in naïve B cells in the context of metabolic disease biases them toward rapid PC differentiation rather than germinal center reactions, leading to loss of both high affinity long-lived PCs and memory B cells. Furthermore, by modulating the adjuvant strength and antigen persistence, we hypothesize that we can tune this response to optimize humoral immune responses in the context of obesity. In our preliminary data, we observed that wild type mice on high fat diet produce fewer long-lived, high affinity PCs upon vaccination. In our future studies we will determine the differentially activated metabolic pathways by RNA-sequencing, as well as perform functional assays to assess cell intrinsic effects of diet induced obesity on B cells. These studies highlight the key role of B cell metabolism plays in the formation and durability of the antibody response to novel vaccination platforms.

58

CAR-T cell targeting three receptors on autoreactive B cells for systemic lupus erythematosus therapy

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by dysregulated B cell activation, autoantibody production, and nephritis. B cell activating factor (BAFF) overexpression enhances autoreactive B-cell survival, driving autoimmunity. BAFF specific Belimumab and CD20 specific Rituximab antibodies are used for SLE therapy, but not curable, suggesting the need for alternative B cell depletion therapies. Here, we used BAFF chimeric antigen receptor T (CAR-T) cells targeting BAFF-R, BCMA and TACI expressed on mature B cells. BAFF receptors are not expressed by early B cells avoiding B cell aplasia and are expressed by long-lived plasma cells avoiding their escape post BAFF CAR-T therapy. We generated murine CD8+ T cells expressing BAFF CAR to test its therapeutic efficacy using spontaneous (MRL/lpr) and pristane induced (BALB/c) mouse models of SLE. In both mouse models, BAFF CAR-T cells treated mice showed persistent killing of mature B cells followed with a decrease in the production of autoantibodies (IgM, IgG and Anti-DNA IgG) and proteinuria along with prolonged survival. The adaptive transfer of B cells from control MRL/lpr lupus mice to previously BAFF CAR-T injected MRL/lpr lupus mice showed continued depletion of B cells and prolonged survival. Lastly, human BAFF CAR-T cells efficiently killed B cells after co-culture with Peripheral Blood Monocytic Cells (PBMC) from active SLE patients and in patient derived SLE xenograft mouse model developed by injecting patient PBMC's to immunocompromised mice. Overall, these data shows a drug free remission of lupus pathogenesis using the BAFF-CAR-T cells suggesting its potential use in SLE therapy.

59

Elucidating inflammatory mechanisms involved in the IMQ-induced psoriasis mouse model: A study on the role of isostearic acid in skin inflammation

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Psoriasis is a chronic immune-mediated skin disease characterized by epidermal hyperplasia and uncontrolled inflammation driven by immune cells including plasmacytoid dendritic cells (pDCs). Topical dorsal skin treatment with imiquimod (IMQ), a toll-like receptor 7 (TLR7) agonist, induces psoriasis in mice similar to human disease. A key limitation of the IMQ-induced psoriasis model is the lack of knowledge about TLR7-dependent and potential TLR7-independent mechanisms by which IMQ induces skin inflammation, particularly the role of the primary vehicle agent, isostearic acid (ISA), in the IMQ (Aldara) formulation. Here, we studied potential TLR7-independent mechanisms that occur in the IMQ-induced psoriasis model and investigated the potential of ISA to induce skin inflammation in mice. Mice treated daily with ISA alone did not develop psoriasis in the absence of IMQ. ISA-treated mice had no changes in body weight and did not develop acanthosis, erythema, scaling and splenomegaly. In contrast, mice treated with IMQ formulated with oleic acid developed classical symptoms of psoriasis. Mechanistically, we found that stimulating immortalized pDCs with ISA had no effect on both NF-κB activation and expression of key inflammatory cytokines, such as TNFα, IL-1β, and IL-6. Taken together, these data suggest that ISA alone has minimal contribution to psoriasis pathogenesis, and the IMQ-induced psoriasis model in mice is primarily driven by TLR7. This greater mechanistic understanding of the specific role of TLR7 in the IMQ-induced psoriasis mouse model is integral in making conclusions from preclinical data and developing clinically translatable therapeutics for psoriasis.

60

Candida tropicalis infection during DSS colitis induces expansion of IL-1β and IL-23-producing NOD2-expressing macrophages

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Inflammatory bowel disease (IBD) is a chronic, relapsing condition of the gastrointestinal tract, resulting from dysregulated gut mucosal immune responses reacting to environmental triggers in genetically-predisposed individuals. IBD patients have an increased presence of *Candida tropicalis* (*Ct*), and the pattern recognition receptor NOD2, which plays a critical role in innate immune responses in IBD, was also shown to be involved in the recognition of fungal wall components. However, how NOD2 regulates mucosal immunity against fungal infection remains unclear. We sought to determine the direct effects of *Ct* on NOD2-expressing macrophages during DSS-induced colitis. Confocal imaging performed on colonic mucosa from WT mice under different experimental conditions (untreated, DSS, *Ct*, DSS+*Ct*) showed that *Ct* induces NOD2-expressing macrophage infiltration into the colonic mucosa of infected DSS colitic mice.To better elucidate the effects of *Ct* on NOD2-expressing macrophages, we isolated bone marrow-derived macrophages (BMDM) from *Nod2*$^{-/-}$ and WT mice and infected them with *Ct*, and/or treated them with fungal cell wall component chitin; cell pellets and supernatants were collected and assessed for macrophage-associated cytokines (IL-1β and IL-23) by qPCR and ELISA. Our results showed a decrease in both IL-1ß and IL-23 in *Nod2* deficient mice compared to WT, and *Il1b* was also markedly decreased in *Nod2*$^{-/-}$ BMDMs infected with chitin and *Ct*. Finally, confocal imaging showed that WT BMDMs were more activated, showing increased patterns of IL-1ß and IL-23 staining, compared with *Nod2*$^{-/-}$ BMDMs. Taken together our data show that macrophages play a key role in NOD2-mediated immune response to *Ct* infection.

61

Microbial influence on sex-based differences in a Crohn's Disease-like ileitis model

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Inflammatory bowel disease (IBD), encompassing ulcerative colitis and Crohn's disease (CD), is a chronic gastrointestinal disorder with an unknown etiology. Dysregulated immune responses, influenced by genetic factors and environmental triggers, notably the gut microbiome, contribute to IBD pathogenesis. Sex-based differences are evident, with females often experiencing earlier onset and more severe disease. Utilizing the SAMP1/YitFc (SAMP) mouse model, our study aims to elucidate the interplay between gut microbiota and host immune responses in IBD. Preliminary 16S rRNA sequencing results revealed pronounced sex-based disparities, with increased α-diversity in specific pathogen-free (SPF)-SAMP and divergent β-diversity trends between SAMP and control AKR mice. Moreover, there was notable β-diversity divergence between SAMP females and males over time. Germ-free (GF)-SAMP still developed ileitis but with attenuated inflammation and delayed disease onset, abolishing sex-based differences observed in SPF conditions. Additionally, SPF-SAMP-M exhibited increased CD4+Foxp3+ Treg frequency compared to females, a phenomenon abolished under GF conditions. Immunophenotyping showed significant cytokine profile differences between GF and SPF conditions, with Th17-associated cytokines elevated in SPF mice, particularly IL-17A and IL-22 in SPF-SAMP-F. Fecal microbiota transplantation from SPF-SAMP-F donors induced more severe colitis in DSS-treated GF-SAMP recipients, regardless of sex, suggesting a 'microsexome' influence on IBD outcomes. These findings underscore the role of microbiome-mediated sex-based differences in IBD.

62

Evaluating the invasion of erythroid lineage cells by *Plasmodium vivax* and *Plasmodium knowlesi* (PvDBPOR) transgenic parasites

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Plasmodium vivax (*Pv*) induces blood stage infections by invading red blood cells (RBCs) using the Duffy (Fy) blood group antigen system. Fy blood group-positive (Fy+/+ or Fy+/-) individuals are susceptible to infection, while Duffy-negative (Fy-/-) individuals show resistance. Recent studies challenge this paradigm by reporting that Fy-/- populations across sub-saharan Africa show incidences of *Pv* infection. Studies suggest that human bone marrow (hBM) may act as a biological niche for *Pv* invasion into reticulocytes (the parasite's preferred target cells), but this has not been substantiated. To investigate this, a reliable culturing protocol and method of assessment is required. Research shows that culturing *Pv in vitro* is not only challenging, but impossible on a long-term scale, necessitating more innovative methods. We demonstrate that hBM can be utilized to culture *Pv* and propagate the parasite on a long-term basis (over 1 year) for a series of experiments. In addition, imaging flow cytometry (IFC) provides a reliable method to monitor and analyze *Pv* cultures by fluorescence-labeling and visual inspections of individual cells to accurately distinguish parasite invasion into hBM. To address our limitations, we have introduced the more easily cultured *P. knowlesi* (Pk) that had been genetically modified to replace its endogenous Fy binding protein (DBP) orthologue with PvDBP to create a transgenic parasite, PkPvDBPOR. This transgenic parasite requires Fy to invade human erythrocytes but is not reticulocyte restricted. Using this model, we evaluated the culture under different perturbations (e.g. Fy-specific antibodies) and discovered low level *in vitro* invasion of Fy-/- recipient cells.

63

Gasdermin B (GSDMB) associates with –and regulates– GSDMD in intestinal-derived goblet cells and is upregulated in inflamed tissues of ulcerative colitis (UC) patients

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Gasdermins (GSDMs) are originally described for their role in pyroptosis. Dysregulation of GSDMs have been observed in chronic inflammatory disorders, including inflammatory bowel disease (IBD). Our group described increased GSDMB in ulcerative colitis (UC) patients, in colonocytes/crypt top colonocytes; however, significant increase in *GSDMB* was also observed in goblet cells (GCs). We aimed to confirm the presence and functional consequences of increased GC-derived GSDMB in IBD. GO analysis was performed on *GSDMB*-expressing GCs from scRNA-Seq-derived intestinal epithelial cells (IEC) from UC patients and healthy controls, and co-expression of other GSDMs evaluated. Based on these results, further analysis was performed on GCs positive for both *GSDMB* and *D*. Using the LS174T GC cell line, regulation of GSDMB and D was determined, and co-localization/proximity evaluated using Duolink®. Our results showed *GSDMB* and *D* overexpression in GCs from inflamed areas of UC patients, with enriched pathways associated with antigen processing/presentation, protein folding, ER stress, but none with pyroptosis or cell death. Co-localization of GSDMB and D was observed in a subpopulation of GCs in UC-derived colonoids and confirmed in LS174T cells. Increased full-length, but not cleaved, GSDMB and D were detected in GCs after IFNg stimulation, with accumulation in the cytoplasm, but with sparse translocation to the plasma membrane. Finally, GSDMB-dependent GSDMD regulation was noted comparing IFNg-treated WT vs. *GSDMB*$^{-/-}$ IECs. Our results indicate GSDMB/GSDMD upregulation and co-operation in GCs derived from active UC, implying an important role in mucin secretion and potential downstream effects in the pathogenesis of IBD.

64

Potent AMA1-specific human monoclonal antibody against *Plasmodium vivax* pre-erythrocytic and blood stages

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One-third of the human population is at risk of contracting *Plasmodium vivax* (Pv) and development of novel Pv-specific therapeutic options is vital in order to decrease the overall disease burden worldwide. Apical Membrane Antigen 1 (AMA1) is a protein that is expressed and utilized by both sporozoites and merozoites during host cell invasion. PBMCs from a Pv-exposed individual were screened for the presence of AMA1-RON2 blocking antibodies. Twelve human monoclonal antibodies (humAbs) were isolated, produced, and the functional characteristics were analyzed. One humAb, 826827, blocks invasion of human reticulocytes using Pv clinical isolates in vitro (IC_{50}= 48 µg/mL). 826827 also inhibited sporozoite invasion of a human hepatocyte cell line and primary human hepatocytes (IC_{50} = 0.3 – 3.7 µg/mL). The crystal structure of recombinant PvAMA1 with the antigen-binding fragment of 826827 shows 826827 partially occupies the highly conserved hydrophobic groove in PvAMA1 that binds its known receptor, RON2. Competition ELISAs confirm that 826827 competes with RON2 peptide for AMA1 binding with a higher affinity, accounting for its potency. *In vivo* testing of 826827 using FRG chimeric mice that allow for a sustained liver stage Pv infection show a marked reduction in parasite burden. 826827 binds to highly conserved residues on PvAMA1, explaining its strain transcending properties. To our knowledge, 826827 is the first humAb reported that is specific to PvAMA1. Additionally, 826827 is one of the first AMA1-specific antibodies that has shown potent inhibition against both pre-erythrocytic and blood stages of Pv *in vitro*.