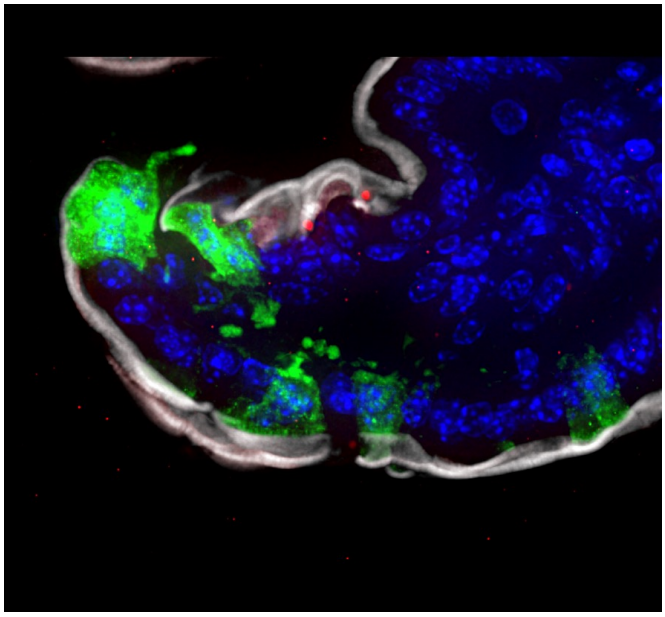
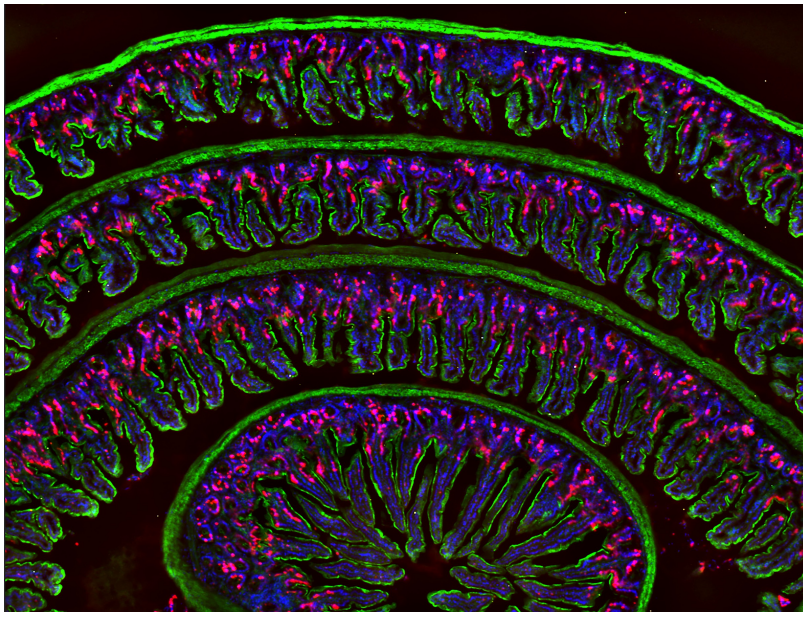
INSTITUTE FOR IMMUNOLOGY

“Penn Immunology: Year in Review”

ABSTRACT BOOKLET





INSTITUTE FOR IMMUNOLOGY

Presents:

"Penn Immunology: Year in Review"

ABSTRACT BOOKLET

Table of Contents

Penn Immunology: Year in Review

Thursday, May 26th | 11:00 AM – 5:30 PM

Smilow Center for Translational Research

11:00 AM – 11:15 AM	**Introduction by** John Wherry, PhD, Director, IFI Warren Pear, MD, PhD, Deputy Director, IFI
11:15 AM – 12:00 PM	**Immune Health Overview** Allie Greenplate, PhD - Associate Director, Immune Health Project *"Immune Health: Broad and Deep Immune Profiling to Improve Patient Care"* Amit Bar-Or, MD, FRCPC – Melissa and Paul Anderson President's Distinguished Professor *"Implementing lessons from immune perturbation with RNA vaccine in multiple sclerosis patients on B-cell depleting therapy"*
12:00 PM – 1:20 PM	**Lunch**
1:20 PM – 1:40 PM	**2022 Immunology Graduate Group Saul Winegrad Awardee** Claudia Arevalo, PhD *"Of Mice, Ferrets, and Men: From Original antigen sin to a new universal influenza virus vaccine candidate"*
1:40 PM – 2:30 PM	**Introduction by** Jon Epstein, MD - Executive Vice Dean and Chief Scientific Officer **Roberts Family-Katalin Karikó Fellowship Program** Michela Locci, PhD – Assistant Professor of Microbiology *"Immunological mechanism of mRNA vaccines"* Norbert Pardi, PhD – Assistant Professor of Microbiology "Development and evaluation of lyophilized nucleoside-modified mRNA-LNP vaccines"
2:30 PM – 3:15 PM	**Matis Family IFI Investigator Award** Katalin Karikó, PhD - Adjunct Professor in Neurosurgery, Senior Vice President at BioNTech *"Developing mRNA for therapy"* Drew Weissman, MD, PhD - Roberts Family Professor in Vaccine Research *"Nucleoside-Modified mRNA-LNP Therapeutic"*
3:15 PM – 3:30 PM	**Break**
3:30 PM – 4:30 PM	**Keynote Presentation:** Erika Pearce, PhD - Bloomberg Distinguished Professor, Johns Hopkins School of Medicine *"Mitochondrial Shape-Shifting in the T Cell Response"*
4:30 PM – 5:30 PM	**Poster Session/Reception**

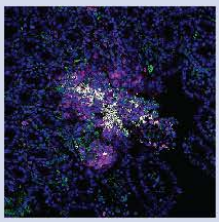
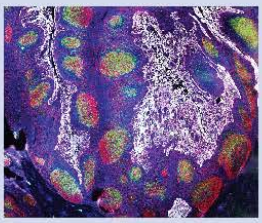
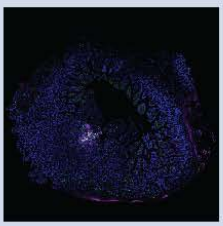
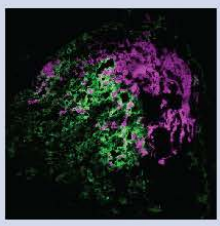





Image descriptions and credits, left to right, Granuloma zoom (Brodsky Lab), Human tonsil, in 40 colors (Wherry Lab), Granuloma (Brodsky Lab), Yersinia Peyers Patch (Brodsky Lab)

2022 IFI Faculty Recognition

The **Matis Family Investigator Award**, established in 2019 through a generous gift from Drs. Louis and Lyn Matis, recognizes and supports outstanding research led by faculty in the Penn Institute for Immunology. We are deeply grateful to Drs. Louis and Lyn Matis for their philanthropic support that confers a distinguished honor on each annual award recipient and recognizes our faculty investigators' most exciting and bold work. The 2022 Matis Family Investigator Award is being presented to Katalin Karikó, PhD and Drew Weissman, MD, PhD for their pioneering work on mRNA vaccines.

The **Roberts Family-Katalin Karikó Fellowship in Vaccine Development** supports early career investigators in the Penn Institute for Immunology, enabling recipients to expand their critical vaccine research in honor of Dr. Karikó's groundbreaking mRNA research conducted in partnership with Dr. Drew Weissman. We thank the Roberts Family and the Aileen K. and Brian L. Roberts Foundation for this generous and meaningful support of the next generation of vaccine researchers, established in 2021. The 2022 Roberts Family-Katalin Karikó Fellowship recognizes Michela Locci, PhD and Norbert Pardi, PhD.

Speaker Bios

Allie Greenplate, PhD – Dr. Greenplate graduated with a bachelor's degree in Biology from the Templeton Honors College at Eastern University in St. David's, PA. She spent 2 years working for Johnson & Johnson, studying antibody responses in cancer and infectious disease, before pursuing her PhD in Microbiology and Immunology at Vanderbilt University. Her thesis work focused on systems immune monitoring in patients receiving anti-cancer treatment, including immunotherapy and small molecule inhibitors. In 2019, Allie came to the University of Pennsylvania to complete a post doc with John Wherry. After helping mobilize COVID-19 research efforts on campus, she transitioned to her current position as the Associate Director of Immune Health. Immune Health acts as a central hub for curating clinical cohorts, performing immune assays, and integrating clinical and immunological data. Immune Health aims to drive new biological discoveries, develop routine clinical immune profiling, and shift paradigms of clinical care.

Amit Bar-Or, MD, FRCPC - Dr. Bar-Or holds the Melissa and Paul Anderson Presidents Distinguished Chair at the University of Pennsylvania and Children's Hospital of Philadelphia, where he Directs the Center for Neuroinflammation and Experimental Therapeutics (CNET) and serves as Chief of the Division of Multiple Sclerosis (MS) and related disorders. His clinical focus is on neuroinflammatory disorders, principally MS, and he runs a cellular and molecular Neuroimmunology lab directed at understanding general principles of immune regulation, immune-neural interactions, and their contributions to inflammation, injury, and repair of the human central nervous system. His studies provide unique windows into the roles of inflammation in normal brain development and across a growing range of neurological, neurodevelopmental, and psychiatric conditions, help elucidate mode-of action of emerging therapies, and develop clinically meaningful biomarkers in advancing precision medicine.

Claudia Arevalo, PhD is a Senior Scientist in the Vaccine Research and Development division at Pfizer, Inc. Dr. Arevalo is a recent graduate of the Immunology graduate program at the University of Pennsylvania. She completed her PhD in Dr. Scott Hensley's laboratory in 2022. Her work explored the phenomenon of original antigenic sin in antibody responses to influenza infection. Additionally, Dr. Arevalo led efforts in developing and testing a novel mRNA-based universal influenza vaccine candidate.

Michela Locci, PhD – Dr. Locci graduated magna cum laude in Biotechnology from the University of Bologna, Italy and received her PhD in Immunology and Applied Biotechnology from Tor Vergata University, Italy. After her postdoctoral training in Dr. Shane Crotty's group, she joined the University of Pennsylvania as an Assistant Professor of Microbiology in 2018. Her research is focused on the biology of T follicular helper (Tfh) cells and regulation of germinal center GC responses and has contributed to our understanding of the nature of memory Tfh cells and the processes regulating the differentiation of Tfh cells. Currently, major efforts in the Locci lab are aimed at shedding light on the quantitative and qualitative features of Tfh and GC B cell responses elicited by messenger RNA vaccines.

Norbert Pardi, PhD – Dr. Norbert Pardi holds a Ph.D. in biochemistry and genetics. He has been working at the University of Pennsylvania since 2011 and currently holds an Assistant Professor position in the Department of Microbiology of the Perelman School of Medicine. His research interest is the development of mRNA-based therapeutics with particular focus on new generation infectious disease vaccines. He explored the development of a novel vaccine platform using nucleoside-modified mRNA in lipid nanoparticles (LNPs) and used it to generate highly effective vaccines targeting various pathogens (influenza virus, coronaviruses, malaria and others). Dr. Pardi is a pioneer of the nucleoside-modified mRNA vaccine technology and published milestone papers in the field.

Katalin Karikó, PhD is a Hungarian-American biochemist who specializes in RNA-mediated mechanisms. Her research has been the development of in vitro-transcribed mRNA for protein therapies. She co-founded and was CEO of RNARx, from 2006 to 2013. Since 2013, she has been associated with BioNTech RNA Pharmaceuticals, first as a vice president and promoted to senior vice president in 2019. She also is an adjunct professor at the University of Pennsylvania. Karikó's work includes the scientific research of RNA-mediated immune activation, resulting in the co-discovery with Dr. Drew Weissman of the nucleoside modifications that suppress the immunogenicity of RNA. This is seen as further contribution to the therapeutic use of mRNA. Together with Weissman, Dr. Kariko holds U.S. patents for the application of non-immunogenic, nucleoside-modified RNA. This technology has been licensed by BioNTech and Moderna to develop their protein replacement technologies but was also used for their COVID-19 vaccines. Dr. Karikó has received many awards, including the prestigious Lasker-DeBakey Clinical Medical Research Award and Time Magazine's Hero of the Year 2021.

Drew Weissman, MD, PhD is a professor of Medicine at the Perelman School of Medicine, University of Pennsylvania. He received his graduate degrees from Boston University School of Medicine. Dr. Weissman, in collaboration with Dr. Katalin Karikó, discovered the ability of modified nucleosides in RNA to suppress activation of innate immune sensors and increase the translation of mRNA containing certain modified nucleosides. The nucleoside-modified mRNA-lipid nanoparticle vaccine platform Dr. Weissman's lab created is used in the first 2 approved COVID-19 vaccines by Pfizer/BioNTech and Moderna. They continue to develop other vaccines that induce potent antibody and T cell responses with mRNA–based vaccines. Dr. Weissman's lab also develops methods to replace genetically deficient proteins, edit the genome, and specifically target cells and organs with mRNA-LNPs, including lung, heart, brain, CD4+ cells, all T cells, and bone marrow stem cells.

Erika Pearce, PhD - Dr. Pearce obtained her Ph.D. in Cell and Molecular Biology in 2005 at the University of Pennsylvania in Philadelphia, where she studied the regulation of T cell responses during infection. During her postdoctoral studies, also at the University of Pennsylvania, she began her research into how cellular metabolic processes govern immune responses to infection and cancer. She launched her independent career in 2009, holding faculty positions at the Trudeau Institute in NY and then Washington University School of Medicine in St. Louis. She moved her research group to Europe in 2015 to become a Director at the Max Planck Institute for Immunobiology and Epigenetics in Freiburg, Germany. In 2018 she was awarded the Gottfreid Wilhelm Leibniz Prize from the DFG for her work on immunometabolism. In 2021 she became a Bloomberg Distinguished Professor at the Johns Hopkins University in Baltimore. Her work continues to investigate the connection between metabolism and cell function.

Trainee Awards & Honors 2021-2022

Sokratis Apostolidis

- 2021 American College of Rheumatology (ACR) Distinguished Fellow Award

William Molina Arocho

- Honorable Mention - Application to the National Science Foundation Graduate Research Fellowship Program

Rishi Goel

- Recipient of the 2022 Paul & Daisy Soros Fellowship Award

Jordan Harris

- F31 NRSA Individual Fellowship Award from NIAMS. Project Title: "The trigger and homeostatic function of a novel immune-sebum circuit".

Zach Lanzar

- 2nd Place Poster Presentation Award at the Woods Hole Immunoparasitology Conference

Olivia Lenz

- Recipient of the Comparative Gastroenterology Society/Royal Canin Professional Development Award

Xin Lin, PhD

- Career Transition Award K99/R00 from NHLBI on 8/5/2021. Project Title: "Mechanisms of GM-CSF-mediated metabolic regulation of monocyte function for control of pulmonary infection".

Jennifer Londregan

- Awarded a T32: Training in HIV Pathogenesis T32 Program

Clara Morral Martinez, PhD

- Recipient of the 2nd best Talk at the BPC Biomedical Postdoc Symposium

Divij Mathew

- Selected as an American Association of Immunologists 2022 Intersect Fellow under the mentorship of John and Dr. Nancy Zhang (Wharton Statistics) to undertake one year of training in computational immunology.

Yuki Muroyama

- Young Investigator Award, Society for Immunotherapy of Cancer (SITC) Abstract Travel Award, SITC 36th Annual Meeting, November 2021
  American Associations for Cancer Research (AACR)-Bristol Myers Squibb (BMS) Scholar-in-Training Award, AACR Annual Meeting, April 2022
- Federation of Clinical Immunology Societies (FOCIS) Annual meeting Travel Award, June 2022
- Japan Society for the Promotion of Science (JSPS) Fellowship, Japanese Ministry of Education and Science, 2021

Derek Oldridge, MD, PhD

- Selected as a Parker Bridge Fellow

Jay Ortiz

- Selected as the IDEAL Council Chair in GAPSA (grad student government)
- Awarded the NASEM Ford Predoctoral Fellowship, nominated for the Blavatnik Fellowship
- Presented with the 2022 AAI-Thermo Fisher Trainee Achievement Award at the Immunology 2022 meeting.

Stephanie Schreiner

- Recipient of the NSG GRFP (National Science Foundation Graduate Research Fellowship Program)

Christine Vazquez

- 2021 - Recipient of the Burroughs Wellcome PDEP Award
- 2022 - Selected as a Leading Edge Fellow

Jason Xu

- Awarded an F31

Faculty Awards & Honors 2021-2022

Michael Abt, PhD
- ACD Spatial Genomics Validation Pilot Award - Advanced Cell Diagnostics Bio-Techne/UPenn

Daria Babushok, MD, PhD
- ASH Scholar Award: ASH Scholar Award Recipients

Amit Bar-Or, MD, FRCP
- Barancik Award for Innovation in Multiple Sclerosis

Daniel Beiting, PhD
- Jane M. Glick Graduate Student Teaching Award

Edward Cantu, MD
- 2020-Present - Board of Directors, International Society for Heart and Lung Transplantation
- 2020-Present - Acute to Chronic Pain Signatures (A2CPS) Expert Panel, NIH
- 2021-Present - Organ Recovery Collaborative Network Standards and Quality Assurance Committee, American Society of Transplant Surgeons
- 2021-Present - ISHLT Membership Task Force (Chair), International Society for Heart and Lung Transplantation
- 2022-Present - OPTN Regional Representative Thoracic Committee (Lung)

Youhai Chen, MD, PhD
- Elected to the 2022 Class of the AIMBE College of Fellows

Jason Christie, MD
- Named president elect of the International Society for Heart and Lung Transplant

Antonella Cianferoni, MD, PhD
- The First Italian American Medical Award, for her work on Food Allergies and Eosinophilic Esophagitis

Cesar de la Fuente Nunez, PhD
- 2021 - Young Innovator in Cellular and Molecular Bioengineering
- 2021 - Biomedical Engineering Society (BMES) CMBE Rising Star Award
- 2021 - Galician of the Year- El Correo Gallego
- 2021 - Philadelphia Business Journal 40 Under 40
- 2021 - Edwin Schultz Lecture, Stanford University
- 2021 - ASM Distinguished Lecturer
- 2021 - Waksman Foundation Lecturer
- 2021 - AIChE Delaware Valley Section (DVS) Outstanding Faculty Award
- 2021 - Princess of Girona Prize for Scientific Research
- 2021 - IADR Innovation in Oral Care Awards
- 2021 - CSM Thermo Fisher Award
- 2021 - Elected member of the Young Academy of Spain

- 2021 - IEEE EMBS Academic Early Career Achievement Award “For the pioneering development of novel antibiotics designed using principles from computation, engineering, and biology.”
- 2021 - Antibiotics 2020 Young Investigator Award
- 2021 - Contributing expert for REVIVE Advancing Antimicrobial R&D at Global Antibiotic Research and Development Partnership (GARDP)
- 2021 - 2022 ASM Award for Early Career Applied and Biotechnological Research
- 2021 - Forbes top 100 most creative Spanish individuals
- 2021 - Innovator Award from Diario de León
- 2021 - Clarivate Highly Cited Researcher
- 2021 - Forbes Top 50 Awarded Spaniards
- 2021 - Early Career Investigator Ad Hoc Council Participant at NIGMS NIH
- 2022 - IJMS 2021 Young Investigator Award
- 2022 - Judge. MIT Technology Review 35 Innovators Under 35.
- 2022 - Young Investigator Award from the Royal Spanish Society of Chemistry (RSEQ)

Laurence (Ike) Eisenlohr, VMD, PhD

- CHOP Academic Training Advisory Council (ATAC)

Hildegund Ertl, MD

- Received funding for the development of COVID-19 vaccine from, The G. Harold and Leila Y. Mathers Charitable Foundation, the Commonwealth of Pennsylvania, the Wistar Science Discovery Fund and a fellowship for a postdoc from Janssen Scientific Affairs.

Joel Gelfand, MD

- 2021 - Article selected as "Best of the best in psoriasis" by JAAD (The risk of RTI psoriasis patients treated with IL 17 biologics: A meta-estimate relevant to decision making during the COVID-19 pandemic)
- 2021 - Best of the Best, "National Psoriasis Foundation COVID-19 Task Force Guidance for Management of Psoriatic Disease During the Pandemic: Version 1"
- 2021 - James J Leyden Endowed Professorship in Clinical Investigation, Inaugural Chair Awardee
- 2022 - Founders Award, American Dermatoepidemiology Network
- 2022 - Top Doctor, Philadelphia Magazine

George Hajishengallis, DDS, PhD

- Designated Highly Cited Researcher by Clarivate/Web-of-Science in 2021

De’Broski Herbert, PhD

- Penn Presidential Associate Professor Award
- Promoted to Penn Presidential Professor

David Hill, MD, PhD

- Hartwell Investigator and received the Hartwell Individual Biomedical Research Award

Alex Huang, MD

- W.W. Smith Charitable Trust Award
- Pew Stewart Scholars Award

Ning (Jenny) Jiang, PhD

- 2021 - Elected to American Institute of Medical and Biological Engineering
- 2021 - Cancer Research Institute Lloyd J. Old STAR award

Chengcheng Jin, PhD

- Damon Runyon-Rachleff Innovation Award

Kellie Jurado, PhD

- 2022 - ICIS-Christina Fleischmann Award for Excellence in Cytokine & Interferon Research
- 2022 - Elizabeth Bingham Award from the Philadelphia Chapter of the Association for Women in Science
- 2022 - National Postdoctoral Association Gallagher Mentor of the Year

Rahul Kohli, MD, PhD

- Dean's Award for Excellence in Basic Science Teaching this year from PSOM

Maayan Levy, PhD

- 2021 – Recipient of the Borrelli Family Pilot Grant in Lynch Syndrome Award

Michela Locci, PhD

- 2021 – Co-Recipient of the Roberts Family - Katalin Karikó Fellowship Program in Vaccine Development Award

Nuala Meyer, MD

- 2022 - Co-awarded the Donald B. Martin Teaching Award from the Dept of Medicine Internal Medicine residency

Michael Mitchell, PhD

- 2022 - NSF CAREER Award
- 2022 - National Academy of Medicine Emerging Leaders Forum
- 2022 - Society for Biomaterials Young Investigator Award
- 2021 - Emerging Inventor of the Year, Penn Center for Innovation
- 2021 - Inaugural Rising Star Award, Journal of Nanobiotechnology
- 2021 - 40 Under 40 Alumni Award, Stevens Institute of Technology

Norbert Pardi, PhD

- 2021 - BIAL Award in Biomedicine (international)
- 2021 – Co - Recipient of the Roberts Family - Katalin Karikó Fellowship Program in Vaccine Development Award
- 2021 - Dennis Gabor Inventor Award (international)

Aimee Payne, MD, PhD

- 2021 Recipient of the Eugene J. Van Scott Award for Innovative Therapy of the Skin

Daniel Powell, PhD

- 2021 Recipient of the Thomas Jefferson University JCLS Distinguished Alumni Award

Alain Rook, MD

- The Herschel Zackheim lecture “My impressions of the role of immunotherapy for cutaneous T-cell lymphoma spanning the past 35 years” Presented at the United States Cutaneous Lymphoma Consortium Annual Meeting, Boston MA.
- Recipient of the Eugene Van Scott Award and presented the Philip Frost lecture entitled “Harnessing Innate Immunity to Treat Cutaneous T-Cell Lymphoma” at the Annual American Academy of Dermatology Meeting, Boston, MA.

Marco Ruella, MD

- 2021 - ISNAFF mentor (Mentee: TBD)
- 2021 - Leukemia and Lymphoma Society, Translational Research Program (TRP)
- 202 - Leukemia and Lymphoma Society and Hairy Cell Leukemia Foundation, HCL2020-25 program Translational Grants
- 2022 - Alan Steinberg Scholars in Cancer Research Award

Carla Scanzello, MD, PhD

- Starting as Associate Editor, Arthritis & Rheumatology
- Dean's Award for Excellence in Clinical Teaching at an Affiliated Hospital, University of Pennsylvania Perelman School of Medicine
- Invited Participant, NIH Roundtable, “Inflammation Resolution: Emerging Opportunities for the NIAMS Mission”

Kathleen Sullivan, MD, PhD

- CIS Presidential Award in 2022

Sarah Tishkoff, PhD

- National academy of medicine and the American Academy of Arts and Sciences

Laura Vella, MD, PhD

- Doris Duke Clinical Scientist Development Award

Robert Vonderheide, MD, DPhil

- Elected, to the board of directors of the American Association of cancer research.
- Appointed, program chair, of the 2023 annual meeting of the American Association of cancer research
- Re-appointed to a five-year term as Director of the Abramson Cancer Center

Evan Weber, PhD

- Steven A Rosenberg Scholars award from SITC

Drew Weissman, MD, PhD

- 2021 - Francis C. Wood Department of Medicine Award for Research, University of Pennsylvania
- 2021 - Wilkins Visiting Professor at the Department of Medicine's Evans Research Days, Boston University School of Medicine
- 2021 - Princess of Asturias Award for Technical and Scientific Research, Princess of Asturias Foundation
- 2021 - The John Scott Medal and Premium, The City of Philadelphia
- 2021 - Inaugural, President's Distinguished Speaker, Association of Senior and Emeritus Faculty, University of Pennsylvania
- 2021 - Inventor of the Year, International Property Owners Education Foundation

- 2021 - Advocacy Award, Research!America
- 2021 - CHAV-ID distinguished scientist, Duke University
- 2021- Lasker~DeBakey Clinical Medical Research Award, Lasker Foundation
- 2021 - Louisa Gross Horwitz Prize, Columbia University
- 2021- Matis IFI Investigator Award
- 2021 - 50th Rosenstiel Award for Distinguished Work in Medical Science, Brandeis University
- 2021 - William B. Coley Award for Distinguished Research in Basic Immunology, Cancer Research Institute.
- 2021 - Brandeis University Alumni Achievement Award, Brandeis University
- 2021 - Alexandra Jane Noble (AJN) Awards, Science Impact Award
- 2021 - Epiphany Award, Science Impact Award. Alexandra Jane Noble for the Novim Group
- 2021 - Albany Medical Center Prize in Medicine and Biomedical Research, Albany Medical Center
- 2022 - Elected into the American Academy of Arts & Sciences
- 2022 - Novo Nordisk Prize, Novo Nordisk Fonden, Denmark
- 2022 - Franklin Institute award, Philadelphia, PA
- 2022 - Prince Mahidol Award, Bangkok, Thailand
- 2022 - Japan Prize, Tokyo, Japan
- 2022 - VinFuture Award, Hanoi, Vietnam

John Wherry, PhD

- 2021 – Recipient of the Stanley N. Cohen Biomedical Research Award, University of Pennsylvania Perelman School of Medicine
- 2021 – AAAS Fellow
- 2021 – Appointed Director, Colton Center for Autoimmunity, University of Pennsylvania
- 2022 – International Society for Advanced Cytometry CYTO Hooke Awardee

Xu (George) Xiaowei, MD, PhD

- Highly Cited Researchers 2021

ABSTRACTS FOR POSTER SESSION:

Allergy, Asthma and Other Inflammatory Diseases

P1 Omeprazole ameliorates barrier dysfunctions caused by IL-13 in three-dimensional esophageal epithelial cultures.

Ravi Gautam and Melanie Ruffner

Background: Eosinophilic esophagitis (EoE) is a chronic allergic disease that is triggered by specific food and characterized by eosinophil-rich multicellular inflammation, and epithelial structural changes. Proton pump inhibitors (PPIs) are extensively used as a first-line EoE therapy and up to 50% of treated patients achieve remission with PPI. Here, we use a three-dimensional epithelial culture model to determine if omeprazole treatment reverses the transcriptomes and epithelial barriers altered by interleukin (IL)-13, a type-2 cytokine that is upregulated in EoE.
Methods: Human immortalized esophageal epithelial cell lines (EPC2) were grown in an air-liquid interface (ALI) culture. The cultures were treated with 100 ng/ml of IL-13 and/or 50 μM acid-activated omeprazole on the 10th and 12th days. Transepithelial electrical resistance (TEER) values were measured on days 7, 10, 12, and 14. On the 14th day, cell lysate was collected in TRI-reagent (TRIzol) for preliminary RNA sequencing to study transcriptomes. To test the effect of PPI treatment on epithelial cell growth and differentiation, cells from treated ALI were extracted and grown in ALI culture again to screen the second-generation effect of IL-13 and omeprazole. No additional treatments were done on the second-generation ALI culture.
Results: IL-13 treatment significantly decreased the TEER value (15% of control, $p < 0.05$) which was mitigated by omeprazole treatment (50% of control). ALI culture treated with IL-13 in the first generation displayed an extremely low mean TEER value (596 Ω*cm2, 25% of control) in the second generation which was restored by omeprazole (2263 Ω*cm2, 100% of control). In PCA analysis, PC1 (64.59%) differentiates IL-13 treated cells and PC2 (10.91%) differentiates omeprazole treatment. We identified 126 genes that were upregulated ($log2FC \geq 0.6$, $p.adj < 0.05$) and 324 genes that were downregulated ($log2FC \leq -0.6$, $p.adj < 0.05$) when treated with omeprazole in addition to IL-13. Functional annotation of these genes reveals positive enrichment of cell cycle checkpoints and negative enrichment of pathways responsible for degradation of extracellular matrix organization.
Conclusion: Omeprazole treatment is associated with specific transcriptional signatures and inhibits IL-13-induced epithelial barrier dysfunction in the ALI model of esophageal epithelium. Our findings also suggest that IL-13 mediated loss of epithelial integrity may persist across multiple cell divisions, and omeprazole plays a protective role.
Keywords: Eosinophilic esophagitis (EoE), omeprazole, interleukin-13, air-liquid interface culture, transepithelial electrical resistance

P2 Sensory neurons shape allergic Type 2 inflammation in the sinonasal tract

Jay Ortiz-Carpena, De'Broski Herbert, Michael A. Kohanski, Andrew E. Vaughan and Noam A. Cohen

Whether neuro-immune interactions regulate homeostasis vs. inflammation within the sinonasal tract is entirely unknown. Transient receptor potential vanilloid 1 ion channel (TRPV1+)-expressing sensory neurons of the trigeminal ganglion (TG) innervate the upper airway near sinonasal tuft cells (STC), a rare lineage of epithelial cells that initiate inflammation in the intestine by secreting pro-Type 2 cytokines. Given the vicinity of STC and TG neurons, we hypothesized that STC function(s) may be at least partially controlled by neuronal inputs. A mouse model of sinonasal allergic inflammation was developed using intranasal administration of a fungal allergen mix (FAM) that mimics key aspects of CRS pathophysiology, including increased numbers of sneezing bouts, STC, eosinophils, and levels of interleukin (IL)-25 in the

sinonasal fluid. Our data show that FAM also evokes the release of neuropeptides substance P and neuromedin U from TG neurons. Also, chemical ablation of TRPV1+ neurons significantly reduces STC expansion and eosinophil accumulation, which collectively suggests that TRPV1+ sensory neurons produce factors that promote STC to release Type 2 cytokines driving allergic disease pathophysiology. Ongoing studies are designed to uncover important insight(s) into how STC/sensory neuron interactions are involved in the pathogenesis of allergic diseases in the sinonasal tract.

P3 Interferon-gamma Impairs Epithelial Barrier Integrity and Potentiates Cytotoxicity in Eosinophilic Esophagitis

Megha Lal and Melanie Ruffner

Background: Eosinophilic esophagitis (EoE) is a chronic, allergic inflammatory disease that is triggered by specific food antigens. Therapeutic options for EoE are nonspecific and consist of avoidance diet, swallowed steroids, and off-label use of proton pump inhibitors. The inflammation in EoE has elevated type 2 cytokines, and efforts have focused on understanding and treating type 2 inflammation in EoE with mixed success. Recent whole tissue RNA-sequencing efforts highlight the upregulation of type I and II interferon response in adult and pediatric EoE patients. However, the role of these non-type 2 inflammatory networks in EoE immunopathology remain unresolved. The aim of this study was to determine the impact of IFN signaling on esophageal epithelium to better understand its role in EoE.
Methods: Biopsy tissue from pediatric patients with active EoE (>60 eosinophils/mm2) and control patients was dissociated and CD45+ cells were removed using negative isolation beads. We performed RNA sequencing to study the transcriptomic profile in the epithelial-cell enriched population. To study the effect of IFNs on human epithelium we used the immortalized human esophageal keratinocyte cell line, EPC2-hTERT. Flow cytometry was used to determine the presence of IFN receptors on epithelial cells. Esophageal organoids were grown for 11 days in Matrigel in with IFN-γ, IFN-α or without stimulation. Organoids were examined for morphologic and functional disruption following IFN stimulation by assessing organoid size, formation rate. Patterns of proliferation (Ki67) and differentiation (involucrin, IVL) were assessed with immunohistochemistry. The air-liquid interface culture (ALI) model was used to test whether IFN stimulation alters epithelial barrier function. Transepithelial electrical resistance (TEER) and FITC-dextran permeation were evaluated to assess membrane permeability. Cytotoxicity often accompanies inflammatory response. Thus, we also performed functional assays assessing cytotoxicity and examined protein expression of relevant markers through immunohistochemistry in epithelial cells in response to IFN treatment.
Results: Differential gene expression distinguished active EoE from healthy controls. Gene set enrichment analysis (GSEA) identified IFN gamma response and inflammatory response as most significant upregulated pathways in the epithelial cell-enriched samples from EoE patients. Over representation analysis (ORA) identified several pathways of interest: proinflammatory pathways, pathways affecting apical junctions and apoptotic pathways. Epithelial cells expressed similar levels of receptors for both IFN-α and IFN-γ by flow cytometry. However, IFN-α stimulation did not affect organoid morphology, formation rate and size. In contrast, IFN-γ stimulation disrupted the proliferation-differentiation gradient in organoids while also significantly reducing the organoid formation (20%, $p < 0.001$) and size (16%, $p < 0.01$). In ALI culture, IFN-γ treatment decreased TEER (70%, $p < 0.001$) and increased paracellular permeability to FITC-Dextran (15 fold, $p < 0.01$). There was a significant increase in apoptosis in IFN-γ treated epithelial cells as confirmed by flow cytometry. Elevated levels of caspase-3 and -8 were seen at 24- and 48-hours post-treatment ($p < 0.01$). Further, the organoids showed increased expression of apoptotic marker (CasP3 and cPARP) and double-strand DNA break marker (H2AX) by immunohistochemistry.
Conclusions: IFN response gene signature are enriched in the epithelium of active EoE. IFN-γ stimulation triggers disruption of epithelial barrier integrity and cytotoxicity, features relevant to EoE immunopathology, in human epithelium. Our findings offer new insights into EoE pathogenesis and might help in improving therapy options for EoE patients

Autoimmunity

P4 Altered 3D chromatin folding dictates the pathogenicity of T cells in Type 1 Diabetic individuals

Aditi Chandra, Naomi Goldman, Sora Yoon, HPAP Consortium, Ali Naji and Golnaz Vahedi

Type 1 diabetes (T1D) is a complex disorder, involving both genetic and epigenetic components. The disease manifests as destruction of pancreatic beta cells mediated by autoimmune inflammatory infiltrate into the pancreatic islets. Recent studies have shown T1D-associated genetic risk variants to be enriched at T cell-specific regulatory elements, suggesting a prominent pathogenic role of these cells in disease symptom. Since it is difficult to access the pathogenic T cells infiltrating the T1D pancreas, our study attempts to characterize chromatin folding patterns in T cells from lymph nodes draining the pancreas.
Transcriptome analysis showed that both CD4+ and CD8+ T cells residing in pancreatic lymph nodes from diabetic individuals have distinct gene expression signatures compared control donors. Differentially expressed genes were enriched in immune system activation pathways. Differentially accessible chromatin regions could also distinguish between control and T1D CD4+ and CD8+ T cells, suggesting prominent role of these cells in T1D pathogenesis. Our main contribution in this study is to generate ultra-deep chromatin contact maps across purified CD4+ and CD8+ cells using HiC. Although T cells from T1D donors did not vary significantly in terms of chromatin compartmentalization and TAD structure, but we observed significant differences in terms of DNA looping associated with key differentially expressed genes. Single-cell multiome analysis showed differential proportions of naïve and memory CD4 and CD8 T cells, between control and T1D donors. These T cell subsets also differed in terms of both gene expression and chromatin accessibility profiles in T1D donor, indicating at the distinctive pathogenic role of these subsets that might be associated with T1D.
Our study thus suggests that changes in chromatin folding events in CD4+ and CD8+ T cells might be guiding their pathogenic phenotype observed in T1D. Combining this dataset with single-cell assays might guide us to identify cell-type-specific aberrant chromatin folding events involved in T1D. This will lead to better understanding of both the most affected cell-type and chromatin regions involved in T1D.

P5 Elucidating the Role of Dynamic X Chromosome Inactivation Maintenance in the Pathogenesis of Systemic Sclerosis

Nikhil Jiwrajka, Katherine S. Forsyth, Amanda Driscoll, Claudia D. Lovell, Natalie Toothacre, Isabel Sierra, Nora Sandorfi, Sylvia Posso, Jane Buckner and Montserrat Anguera

Background: Systemic sclerosis (SSc) is an idiopathic, highly female-biased autoimmune disease in which immune dysregulation is associated with life-threatening, progressive fibrosis of the skin and lungs. The molecular basis of this female bias and its contribution to SSc pathogenesis is unknown. The X and Y chromosomes provide the genetic basis for sex differences, and female (XX) mammals use X Chromosome Inactivation (XCI) to equalize X-linked gene dosage with males (XY). Maintenance of the inactive X chromosome (Xi) is partially achieved through the non-coding RNA known as XIST RNA, which coats the Xi and reinforces transcriptional silencing. T lymphocytes, which are central to SSc pathogenesis, exhibit an unusual, dynamic form of XCI maintenance, in which XIST RNA is initially cytologically absent from the Xi in resting T cells, and subsequently dynamically relocalizes to the Xi upon T-cell activation.
Objective: Because several proinflammatory and profibrotic X-linked genes exhibit increased expression in T cells from SSc tissues, we sought to determine whether impaired dynamic XCI maintenance in SSc T cells contributes to female-biased disease susceptibility.
Results: We show that peripheral blood CD3+ T cells from females with limited cutaneous SSc (n=5) demonstrate impaired dynamic relocalization of XIST RNA relative to age-matched healthy

females (n=3) following T-cell activation. Using CD4cre+ Xist fl/fl (Xist CKO) mice, in which Xist is deleted specifically in T cells during XCI maintenance, we also show that female Xist CKO mice subjected to the bleomycin-induced model of SSc develop greater dermal and pulmonary fibrosis relative to wild-type females.
Conclusions: These data suggest that impaired dynamic XCI maintenance may be a pathologically relevant mechanism of female-biased disease in SSc. Further study will be instrumental in validating these findings and identifying the spectrum of T-cell-specific proinflammatory and profibrotic transcripts that escape from X inactivation and confer female biased fibrotic disease in SSc.

P6 Proteasome inhibition induces p53 driven apoptosis in early activated B-cells

Trini Ochoa and David Allman

Proteasome inhibitors (PIs) are commonly considered for combating plasma cell-mediated pathologies such as multiple myeloma, antibody-mediated autoimmunity, and chronic transplant rejection. However, although PIs are proposed to selectively target plasma cells and activated B cells, a comprehensive picture of the specific cellular targets affected by PIs and the underlying mechanisms remains to be developed. Here, we evaluate the impact of the PI bortezomib (BTZ) on newborn and long-lived plasma cells as well as activated B cells in germinal centers (GCs) and identify distinct mechanisms for BTZ-depletion for each context. Our results reveal that GC B cells and newborn plasma cells are each highly sensitive to BTZ-mediated depletion, whereas many mature long-lived plasma cells are clearly resistant. Furthermore, aided by a simple in vitro plasma cell induction assay, we found that mutation of the ER-stress induced transcription factor CHOP rescues plasma cells from low-dose BTZ, whereas mutation of the tumor suppressor and cell cycle regulator p53 rescues activated B cells from BTZ-induced apoptosis as cells progressed through the G1 to S phase cell cycle check point. We conclude that p53 and CHOP play unique roles in BTZ-induced apoptosis in activated B cells versus newborn plasma cells.

Cancer Immunology and Immunotherapy

P7 Cellular and humoral responses in patients with melanoma on PD-1 immunotherapy following SARS-CoV-2 mRNA vaccination

Sokratis Apostolidis, Paulina Coutifaris, Mark M. Painter, Ajinkya Pattekar, Rishi Goel, Divij Mathew, Wenzhao Meng, Kevin Ng, Bria Fulmer, Angela R. Corrigan, Kendall A Lundgreen, Elizabeth M. Drapeau, Derek A. Oldridge, Josephine Giles, Amy E. Baxter, Sasikanth Manne, Greg L. Szeto, Michelle McKeague, Ahron Flowers, Oliva Kuthuru, Sherea Long, Jeanette Dougherty, Sigrid Gouma, Justine C. Williams, Maura McLaughlin, Sharon Adamski, Alba Grifoni, Daniela Weiskopf, Alessandro Sette, Scott E. Hensley, Paul Bates, Eline T Luning Prak, Laura A. Vella, Giorgos C. Karakousis, Ravi K. Amaravadi, Kristin Kreider, Tara C. Mitchell, Allison R. Greenplate, Lynn Schuchter, Alexander C. Huang, E. John Wherry

Patients with cancer exhibit increased mortality from SARS-CoV-2 infection compared to the general population. SARS-CoV-2 mRNA vaccination in healthy individuals generates effective and long-lasting immune protection against COVID-19. Here, we investigated the longitudinal induction of antigen-specific antibody, B cell and T cell responses in patients with melanoma on anti-PD-1 immunotherapy (aPD-1, n = 44) receiving a 2-dose SARS-CoV-2 mRNA vaccine regimen during 6 distinct timepoints over the course of 9 months. We compared these responses against patients with melanoma on observation (obs, n=5) and against an established cohort of healthy control individuals (HC, n=45) with similar blood draw collection schedules following mRNA vaccination. All individuals included in this study were not previously infected with SARS-CoV-2. After 2 doses of mRNA vaccination, patients on aPD-1 reached levels of anti-Spike and anti-Receptor Binding Doman (RBD) IgG similar to the patients on observation and the healthy controls. However, aPD-1 patients demonstrated a 2-fold reduction in the neutralizing antibody

levels after the second dose compared to healthy individuals. Vaccine-specific memory B cells (mBCs) recognizing Spike and RBD were generated in patients on aPD-1 therapies to an extent equivalent to healthy controls and patients on observation status. The mBCs from aPD-1 patients efficiently recognized the Alpha, Beta and Delta variants of concern (VOC) of SARS-CoV-2 but exhibited diminished recognition of the Omicron variant at 2 months after the 2nd vaccination. This defect improved over time, with mBCs from aPD-1 patients recognizing the Omicron VOC at levels similar to the control groups by 6-9 months following primary vaccination. Finally, using a peptide pool of the wild-type Spike protein, we found that all aPD-1 patients generated antigen-specific CD4 and CD8 T-cell responses following vaccination. Nevertheless, when tested with an omicron-specific peptide pool, the abundance of antigen-specific CD4 T cells, but not CD8 T cells, from patients on aPD-1 immunotherapy was lower than that of patients on observation or healthy controls. These results indicated that patients on aPD-1 immunotherapeutics generate robust humoral and cellular vaccine-specific responses against the wild-type Spike protein following SARS-CoV-2 mRNA vaccination. However, they also demonstrated diminished B cell and CD4 T cell recognition of the immune-evasive Omicron variant. The qualitative defects seen in patients on aPD-1 therapy highlight the importance of the PD-1 pathway in promoting fully functional vaccine responses, suggesting possibly a more pressing need for these patients to receive booster vaccinations.

P8 Trib1 inhibits viral-specific T cell responses during chronic infection by promoting terminal exhaustion

Susie McClory; Oishi Bardhan; Kelly Rome; Warren Pear and Martha Jordan

The ability of T cells to clear chronic infections or cancer is often limited because they become exhausted and lose robust effector function. We previously discovered that conditional deletion (cKO) of Trib1 from T cells during chronic LCMV infection (clone 13) leads to improved CD8+ and CD4+ effector function and decreased viral burden. We now establish that conditional deletion of Trib1 from all T cells during clone 13 infection leads to an expansion of CD8+CX3CR1+KLRG1+ effector-like exhausted T cells (Tex) and improved persistence of a CD8+CX3CR1+KLRG1– transitional Tex subset previously shown to be critical for viral control. Additionally, expansion of Trib1cKO CD8+CX3CR1+KLRG1+ effector-like cells occurs at the expense of CD8+CD101+PD1+CD69+ cells, a short-lived population of terminally exhausted T cells. These findings suggest that when Trib1 is deleted from T cells during chronic infection, the pathway of terminal exhaustion is suppressed in favor of a KLRG1+ effector-like cell fate. Next, we performed CD4 depletion studies in our CD4-Cre Trib1 cKO model as well as adoptive transfer experiments of Trib1-deficient TCR-transgenic P14 CD8+ cells to uncover both CD4-dependent and CD8-intrinsic effects of Trib1 knockout. Together, these data suggest that the effects of Trib1 on CD8 T cell exhaustion are critically dependent on CD4 help. Finally, we demonstrate that Trib1cKO during chronic infection augments anti-PDL1 blockade to improve viral clearance. These findings provide important insights into the role of Trib1 during T cell exhaustion, the role of CD4 help in regulating and augmenting T cell function during chronic infection and suggest that targeting Trib1 signaling could improve the immune response to chronic antigen exposure, as seen in both infection and cancer.

P9 Allogeneic invariant Natural Killer T cells persist in MHC-mismatched immunocompetent dogs

Antoni Rotolo, A. Rotolo , E. C. Whelan , M. J. Atherton , I. Kulikovskaya , M. M. Kim , E. S. Diffenderfer , K. A. Cengel , M. Piviani , K. Coker , R. Duran-Struuck , N. J. Mason

Background. CD1d-restricted invariant Natural Killer T cells (iNKTs) are attractive for allogeneic cancer immunotherapy given that donor iNKTs do not induce graft-versus-host disease (GVHD) and promote anti-tumor immunity.

Methods. We established a method to isolate and expand canine iNKTs ex vivo. iNKTs were characterized by comparative genomics, proliferation and killing flow cytometric assays, Luminex cytokine profiling and single-cell RNA-sequencing. Ex vivo expanded allo-iNKTs with preserved MHC molecules were infused into healthy, MHC-mismatched non-lymphodepleted recipients of 30-33 kg. The safety, immune effects and persistence were monitored by clinical, hematological and biochemical analyses along with flow cytometry and RT-qPCR assays of peripheral blood and bone marrow samples.
We investigated iNKTs in canines, an excellent model for validation of clinical protocols, to inform use in human patients and elucidate safety and persistence in allogeneic settings.
Results. We found that dog and human iNKTs share an identical T cell receptor Va24-Ja18 rearrangement, have highly similar immunophenotypic features, and their transcriptomes largely overlap. Based on such similarities, we successfully used human protocols suitable for GMP-manufacturing to expand canine iNKTs to large scale. Functional immunoassays confirmed preserved CD1d-restricted reactivity, Th1/2 immunomodulatory potential and cytotoxic activity. Similar to human, canine iNKTs were further enhanced via stimulation with glycolipid agonists and CAR engineering.
Adoptive transfer of 4x108 MHC-intact, mismatched allo-iNKTs was well tolerated. No cytokine release syndrome, acute GVHD or other adverse events occurred. Allo-iNKTs induced host regulatory and cytotoxic cells, consistent with simultaneous allo-tolerance and anti-tumor potential. Remarkably, allo-iNKTs persisted for the duration of the follow-up (42 days).
Conclusions. We demonstrated that the dog is a feasible model for clinically relevant allo-iNKT studies. Allo-iNKTs were safe, functional, and persisted longer than in previous murine and human studies, suggesting that MHC-intact allo-iNKTs could be exploited in the human clinic to induce durable allo-tolerance and anti-tumor effects.

P10 **Dual CAR signaling for the control of CD19 expressing tumor**

Divanshu Shukla, Shuguang Jiang, Sasikanth Manne, Colby R. Maldini, Marco Ruella, Saar Gill, E. John Wherry and James L. Riley

Chimeric antigen receptor (CAR) T cells targeting CD19 demonstrate incredible effectiveness in treating B-cell acute lymphoblastic leukemia (B-ALL). However, CD19 negative relapse increasingly recognized as a major problem of immune escape. Here we report that signaling through dual targeting CART cells bearing different co-stimulatory signaling domain (41BB or CD28) improve the effects of CART cells against B-ALL and diminish the risk of antigen loss escape. Furthermore, combining CD19.28ζ CAR and CD22.BBζ CAR induced comparable IL-2, TNF-α and IFN-γ in-vitro against dual-antigen-expressing Nalm6 B-ALL cell line. Whereas in in-vivo study, dual targeting CD19.BBζ-CD22.28ζ CART cells augments anti-leukemic activity against B-ALL and enhances human T cells persistence and mice survival. On the other hand, CD19.28ζ-CD22.BBζ CART cells provide better tumor control against CD19 loss escape disease. In order to know the molecular basis of these differences, we stimulated the CART cells with cell-free ligands to get a precise signal transduction by CARs. We performed the transcriptional profiling of bulk CART cell populations to characterize the transcriptional states of dual targeting CART cells bearing different co-stimulatory domains 4-1BB or CD28 at rest and after activation by triggering their CARs. We demonstrated that genes related to Th17 cell differentiation, Th1 and Th2 cell differentiation, C−type lectin receptor and NF−kappa B signaling pathways enriched in dual CART cells after their respective ligand engagement. Here we identified that expression of IL-23A, known to promote proliferation of memory T cells and T helper type 17 cells shows a substantial high expression in dual CART cells. Taken together, these finding expand our understanding of how CAR signaling domains affect the gene expression profile, functional state and involved in uniquely shape the transcriptional programs of human T cells. This information will provide a great vision in improving CART cells therapies with specific signaling domains to achieve a desired function.

P11 Therapeutic efficacy of Fli1 ablation on anti HIV CAR T-cell effector functions

Sriram Srivatsa and James Riley

CD4 based chimeric antigen receptor (CD4-CAR) T-cells, are documented to alleviate HIV-induced pathogenesis. Our lab demonstrated that CD4-CAR T-cells expressing both second generation 4-1BB CD3z (CD4BBz) and CD28 CD3z (CD428z) CARs, show enhanced effector function, expansion, protection against loss of CD4 T-cells and its memory subsets, compared to third-generation CAR T-cells, despite eventual HIV rebound. Therefore, it is highly imperative to sustain persistence and improve effector function of HIV-specific CAR T-cells.
Fli1 from the ETS transcription factor family, was recently described to augment T-cell effector differentiation in mouse models of infections and cancer. Specifically, murine recipients of Fli1 deficient CD8 cells showed heightened T-cell proliferation, with increased terminal effector cells with production of effector cytokines, without affecting the production of long-term memory CD8+ T-cells.
In our study we observed that Fli1 deficiency improves proliferation and mildly increase in memory T-cell frequency in-vitro. Interestingly, Fli1 ablation reduced effector functions of CAR T-cells against artificial APCs. Further, lack of Fli1 in murine HIV models leads to decrease of T-cell persistence which is accompanied by rapid onset of viremia. Further experiments currently underway will address the mechanisms underlying the observed phenotype.

Immune System Development and Function in Host Homeostasis

P12 Lymphatic endothelial cell intrinsic IKKα in tertiary lymphoid organ development and function

Michelle Cully, Athena Patel, Julianne Nolte, Nipun Jayachandran, Andrew E. Vaughan and Michael May

The non-canonical NF-κB pathway is required for secondary lymphoid organ development and recent evidence suggests that activation of this pathway in lymphatic endothelial cells (LECs) drives lymphoid organogenesis. To study this in detail, we generated mice lacking LEC-intrinsic IKKα, a central kinase in the non-canonical NF-κB pathway. These mice failed to develop lymph nodes and strikingly, we found peri-bronchiolar and perivascular aggregates of immune cells in their lungs that were otherwise healthy and undamaged. Further assessment of these immune cell accumulations revealed them to be lung-associated tertiary lymphoid organs (TLOs). TLOs are ectopic accumulations of immune cells that arise in a range of inflammatory diseases and cancers, but the mechanisms that control their formation and function remain incompletely understood. We determined that these lung-associated TLOs are highly organized, containing a B cell follicle adjacent to a T cell zone, as well as dendritic cells at the T-B interface and CXCL13 expressing stroma underlying the B cell follicle. Importantly, when we infected the mice with influenza virus, they displayed remarkably improved morbidity and mortality compared with littermate controls. From these findings, we hypothesize that targeting IKKα in LECs impairs immune cell egress from the lung leading to the formation of TLOs that can protect against respiratory infection. We speculate that targeting IKKα in LECs to drive TLO formation may be a valuable treatment opportunity against emerging respiratory pathogens.

P13 The plasma cell proteome initiates early in B cell activation in advance of Blimp-1

Brian Gaudette, Brian T. Gaudette and David Allman

Plasma cell (PC) differentiation is a highly regulated process that radically changes the morphology and function of a B lymphocyte to that of a professional protein secretor. It is becoming clear that this process precedes increase in immunoglobulin synthesis rather than reacting to it. We have shown previously that large segments of the PC transcriptome are initiated in an mTORC1-dependent manner prior to both Blimp-1 and Xbp1s expression. Still, the kinetics of induction of the PC proteome remain largely unknown. We performed a whole proteome kinetic analysis of activated Blimp-1-reporter follicular B cells in PC-inductive conditions every 12 hours for three days and observed distinct patterns in induction of the PC proteome. To elucidate transcriptional networks, we performed single cell RNA-seq with the same conditions and correlated transcription factor expression with genes coding for these proteome modules within single cells. We found distinct subgroups within proteome modules corresponding to transcriptional patterns each with a highly correlated transcription factor set. These included robust, transient, Myc-associated factors required for translation initiation; rapid, sustained expression of protein synthesis machinery; and gradual, sustained increases in proteins associated with ER function and secretion. Each of these preceded the induction of Blimp-1 and increase in immunoglobulin protein. Finally, using inducible deletion of Blimp-1 in conjunction with the reporter allele we show that these unique transcriptional and proteomic programs are initiated independently of Blimp-1 expression. Thus, activated B cells prepare for protein secretion by initiating the PC proteome prior to antibody secretion.

P14 Exploring the mechanisms that establish the chromatin state of T cells

Naomi Goldman, Aditi Chandra, Abhijeet R. Patil, Ashley Vanderbeck, Simone Sidoli, Ivan Maillard, and Golnaz Vahedi

Cell fate-specific gene expression programs are established in part by alterations in chromatin accessibility via the action of lineage-determining transcription factors (TFs). Work in our lab has identified that the transcription factor TCF-1—integral for normal thymic development—targets and is essential for the opening of chromatin in T cells1. To further dissect the mechanism through which TCF-1 acts during T cell development we have tested the effect of deletion of multiple different domains spanning the TCF-1 protein – which do not overlap with the DNA binding domain. Utilizing the OP9-DLL1 co-culture system which recapitulates normal thymic development we demonstrated that a mutant TCF-1 lacking a region of high intrinsic disorder led to an early developmental block producing very few early T cell progenitors. To further investigate the importance of this region within TCF-1 we analyzed changes in the transcriptome and epigenetic landscape of these early T cell populations, which indicated the importance of TCF-1's disordered region in the repression of alternative myeloid fates. Intriguingly, the role of this disordered domain in TCF-1 appears to not be conserved post commitment where transcriptional and epigenetic changes due to loss of this domain after the DN3 stage of development do not parallel their role pre-commitment. These data suggest novel mechanisms through which TCF-1 exerts its regulatory role on T cell lineage specification and underlies the importance of the study of lineage defining transcription factor effector domains.

P15 Akkermansia muciniphila influences adaptive immune responses to the microbiota in early life

Sarah Maddux and Michael Silverman

The mucus-degrading microbe Akkermansia muciniphila is a common human commensal that is correlated with better outcomes for conditions ranging from autoimmune diabetes to cancer immunotherapy; it is an immunostimulatory microbe that strengthens the intestinal epithelial barrier and is capable of inducing antigen-specific T cell and antibody responses. However, little is known about the mechanisms by which A. muciniphila influences host immunity and barrier function. Using a defined murine pediatric microbial community our lab developed, we have found that in addition to inducing peripheral Tregs and stimulating a strong systemic antibody response to itself, A. muciniphila enhances systemic antibody responses to other commensal microbes. These effects only appear when exposure happens prior to weaning in NOD mice, emphasizing the importance of early life microbial exposure in long term immune development. Using a mutant strain of A. muciniphila that cannot degrade mucus, we have also recently found that the mucin-degrading capacity of A. muciniphila is essential for A. muciniphila to elicit a humoral response. Collectively, this data elucidates many of the host and microbe parameters that are necessary for A. muciniphila to influence adaptive immunity.

Immunometabolism

P16 Mitochondria Cristae disturbance promotes T cell activation

Edmund Carvalho, Irene Su, Christopher Ecker, Sriram Srivatsa and James Riley

T cells switch metabolic states from predominately oxidative phosphorylation to aerobic glycolysis to balance biosynthesis and bioenergetics during clonal expansion in the presence of antigen. Our studies demonstrate that mitochondrial cristae disturbance is a key event during mitochondrial biogenesis post antigen stimulation. Elevated cytosolic calcium (cCa2+), responsible for aerobic glycolysis causes cristae disturbance. This cristae disturbance although mediated by calcium, is importantly determined by calcium induced metabolic switch to aerobic glycolysis and increased mitochondrial energetics. Altering glucose and glutamine availability and signaling but not fatty acid oxidation attenuates cristae disturbance suggesting a greater dependence of cristae disturbance on Warburg metabolism induced due to T cell activation. Thus in doing so cristae disturbance reduces the mitochondrial contribution of redox intermediates to match T cell activation. Hence, mitochondrial cristae dynamics is an active component in T cell activation.

Innate and Adaptive Immunity to Pathogens

P17 Skin sensory neurons downregulate myeloid-derived IL-33 for IL-17-dependent immunity against Schistosoma mansoni.

Juan Inclan Rico, Christopher F. Pastore, Li-Yin Hung, Heather Rossi, Annabel Ferguson, Camila Napuri and De'Broski R. Herbert

Interleukin 33 (IL-33) is considered an alarmin cytokine produced by damaged structural cells at barrier sites including the skin to elicit immune responses against injurious substances. However, it is becoming increasingly clear that IL-33 is expressed by diverse cellular sources that mediate divergent biological functions, obscuring our understanding of how IL-33 production is regulated. This work demonstrates that IL-33 was expressed by both hematopoietic and non-hematopoietic cell lineages at baseline, most notably in cutaneous dendritic cell (DC) and macrophage subsets. Our data show that selective deletion of IL-33 in CD11c-expressing cells resulted in a basal increase of dermal IL-17/IL-23 and γδ T cell responses accompanied by increased keratinization and epidermal thickening. Single-cell RNAseq analysis revealed that myeloid-specific IL-33

deficiency increased cell intrinsic expression of IL-17 inducing cytokines (e.g.IL-12p40, IL-18 and IL-1β,) in DC and macrophage subsets under steady-state conditions. This phenotype was consistent with enhanced IL-17 or IL-23-dependent resistance to percutaneous infection with the helminth Schistosoma mansoni, in mice with myeloid-specific IL-33 deficiency, as compared to their littermate controls. Unexpectedly, regulation of myeloid derived IL-33 was dependent upon skin sensory neurons, inasmuch as neuron activation using optogenetics selectively reduced IL-33 protein content in skin cDC2 and tissue macrophage subsets, accompanied by increased IL-17-expressing γδ T cell responses and resistance to S. mansoni infection. Moreover, stimulation of bone marrow derived macrophages with supernatants derived from activated neurons caused a reduction in IL-33 protein content and increased pro-inflammatory cytokine secretion. Overall, these data support a hypothesis that sensory neurons can curtail IL-33 expression in myeloid cells to unleash their cytokine secretion that promotes the expansion of IL-17 producing γδ T cells for host protective immunity against skin-penetrating parasites.

P18 RIPK1 is dispensable for apoptosis in human macrophages in response to Yersinia blockade of immune signaling

Neha Nataraj, Igor E. Brodsky and Sunny Shin

Many microbial pathogens suppress the pro-inflammatory response of innate immune cells to evade detection by their hosts. The gram-negative bacterial pathogen Yersinia, which causes diseases from self-limiting gastroenteritis to systemic bacteremia, utilizes a type III secretion system (T3SS) to inject effectors into host cells. These effectors disrupt NF-kB signaling, thereby preventing activation of cell-intrinsic cytokine production and anti-microbial defense. We and others have demonstrated that in murine cells, Yersinia blockade of NF-kB signaling triggers apoptosis, involving engagement of Receptor Interacting Serine-Threonine Protein Kinase 1 (RIPK1) and caspase-8 (Casp8). In mice, RIPK1-dependent apoptosis is required for bacterial clearance and host survival, as macrophages lacking RIPK1 kinase activity or Casp8 are defective for Yersinia-induced cell death, and the corresponding mice cannot control Yersinia tissue burdens and succumb to infection. While mice express a single Casp8 protease, humans express two orthologs, CASP8 and CASP10. Moreover, while RIPK1- or Casp8-deficient mice undergo embryonic lethality, RIPK1- and Casp8-deficient humans are born but experience autoinflammatory phenotypes accompanied by increased susceptibility to a variety of viral and bacterial infections. Here, we find that both Casp8 and 10 are activated during apoptosis of human macrophages induced by NF-kB signaling blockade. Unexpectedly, in contrast to murine macrophages, human macrophages did not require RIPK1 kinase activity or RIPK1 itself to undergo apoptosis in response to Yersinia-mediated blockade of NF-kB signaling. Rather, we find that the expression of the pro-survival protein, cellular FLICE-Like Inhibitory Protein (cFLIP), is downregulated during NF-kB blockade, and that the absence of cFLIP potentiates RIPK1 kinase-independent apoptosis in response to TLR or TNFR stimulation alone. Altogether, our data indicate that bacterial blockade of NF-kB triggers a human-specific apoptosis pathway in macrophages that is independent of RIPK1, activates Casp8 and 10, and is likely regulated by cFLIP.

P19 Elucidating neuronal innate immune mechanisms in the central nervous system

Seble Negatu and Kellie Jurado

Neurotropic viruses are the most common cause of infectious encephalitis. Published studies have increased our understanding of how resident glial cells and infiltrating leukocytes contribute to neuroimmune responses during pathogen invasion. However, neuron-specific immune responses remain poorly described. Neurons are readily infected by many neurotropic viruses, but studies show neuronal stimulation by viral components poorly induces pathways that are critical for viral control, such as the type I interferon (IFN) response. This leads to our central question of how do neurons orchestrate effective antiviral immunity during CNS infection? To

probe type I IFN responses in the CNS, I have developed an intracranial neonatal infection model using La Crosse Virus (LACV), an emerging RNA virus. Preliminary data show that infections of C57Bl/6 wild-type, Type I IFN receptor (IFNAR) receptor knock-out (KO), and non-canonical type I IFN pathway (STING) KO neonates with both wild-type LACV and LACV-ΔNSs (lacks the known type I IFN antagonist, NSs) viruses causes severe disease and reduced survival of neonates. IFNAR KO and STING KO mice succumb to disease earlier than C57Bl/6 mice. Additionally, in all genetic backgrounds, viral titers have an inverse relationship with survival. Future work will assess cell-specific infectivity of LACV and type I IFN responses in the CNS. I have also developed an in vitro infection pipeline where primary neurons isolated and cultured from mice are infected and further characterized using fluorescence in situ hybridization and RNA-sequencing. I plan to infect neurons of differential maturation states with LACV-ΔNSs and evaluate changes in gene expression compared to uninfected, bystander cells.

P20 Clostridioides difficile in early life

Alexa Semon, Orlaith Keenan, Chao Di, Jonathan Specker, Boone Prentice, Joseph Zackular

Abstract: Early life is a critical developmental window during which a beneficial relationship between the host and its resident gut microbiota is established. However, it is not well understood whether the presence of pathogenic microbes in the gastrointestinal tract early in life is consequential to immune development and long-term health. One of the most important and ubiquitous enteric human pathogens in children and adults is Clostridioides difficile. In adults, C. difficile infection causes a spectrum of disease, including mild diarrhea, pseudomembranous colitis, toxic megacolon, and death. Surprisingly, infants colonized with C. difficile present as asymptomatic, despite being colonized with high burdens of toxigenic strains; thus, making C. difficile a highly relevant clinical example of early life pathogen-immune imprinting. Using a neonatal mouse model, we demonstrate that neonatal mice survive a lethal infection with C. difficile. Following early life colonization, we observe neutrophil recruitment into the colonic lamina propria and marked changes in the succession of the early life microbiota and metabolome. Disruption of the microbial ecosystem early in life is associated with increased susceptibility to inflammatory and metabolic disorders later in life, so we postulated that C. difficile-mediated disruption of the immune-microbiota axis may alter long-term health. We find that mice pre-colonized with C. difficile during early life develop more severe chemical-induced colitis in adulthood compared to C. difficile naïve mice. In conclusion, our data demonstrate that early life colonization with C. difficile has ecological and immunological effects which ultimately affects long-term health by exacerbating inflammation. Furthermore, our data suggest that neonatal colonization with C. difficile may not be immunologically silent and thus has significant implications to both neonatal and adult health.

P21 Enterovirus-D68 regulation of immune responses for neuroinvasion

Christine Vazquez and Kellie Jurado

Enterovirus-D68 (EV-D68) is an emerging respiratory virus that has the capacity to enter and infect cells of the central nervous system (CNS). Though EV-D68 was first identified in the 1960s, it has only recently received attention due to a correlative rise in cases of acute flaccid myelitis (AFM) in children under the age of five. AFM is a severe neurological illness affecting spinal cord motor neurons, leading to motor impairment, muscle weakness, and paralysis. While the etiological agent of AFM remains inconclusive, EV-D68 has emerged as the likely infectious candidate. The prototypic EV-D68 strain, Fermon, is considered non-neuroinvasive as it has limited replication in neuron-like cells, such as the neuroblastoma cell line, SH-SY5Y. However, since 2014, circulating contemporary EV-D68 strains, including US/MO/14-18947 (MO), have caused several outbreaks linked with a rise in AFM cases throughout the US, illustrating neuroinvasive potential. AFM is a rare infection outcome, yet a large percentage of adults express EV-D68 antibodies, implying a likely previous viral exposure, which suggests that host immune responses may play a role in limiting viral spread into neurons. Thus, we hypothesize

that neuroinvasive EV-D68 strains may impede immune signaling to enter the CNS and cause neurological disease compared to the non-neuroinvasive Fermon strain. To identify genes that are differentially expressed upon infection with neuroinvasive (MO) and non-neuroinvasive (Fermon) EV-D68 strains, we performed bulk RNA-sequencing on SH-SY5Y cells and rhabdomyosarcoma muscle cells infected with either MO or Fermon. Our preliminary data suggest that interferon pathway signaling genes are lower expressed in MO-infected SH-SY5Y cells compared to Fermon-infected SH-SY5Y cells. These data imply that neurotropic EV-D68 may antagonize immune genes to enter the CNS. Future experiments will define the specific differentially-expressed immune genes targeted by neuroinvasive EV-D68 strains to enter the CNS and the mechanisms by which they regulate immune responses compared to the non-neuroinvasive Fermon strain.

P22 HIV specific CAR-T cells can kill targets upon priming

Yuqi Zhou, Julie Jadlowsky, Max W. Richardson, Caitlin Baiduc and James L Riley

Adoptive T cell therapy has shown significant progress in treating different types of blood cancer. Its ability against persistent antigens makes it a promising therapy to treat chronic viral infections such as HIV. HIV patients can now live normally with antiretroviral therapy (ART). However, latently infected cells which harbor silent, replication-competent copies of HIV will periodically become active and causes persistent infection of the virus. By adoptively transferring engineered HIV-specific T cells, it is hypothesized that the transferred cell can quickly limit the virion once the latent virus is reactivated, thus reaching a functional cure for the patients. Chimeric antigen receptor (CAR) and TCR αβ chains are the most common antigen recognition molecules engineered into T cells for redirection in adoptive T cell therapy. Previous data from our lab has shown superior HIV suppression in vitro by CD4 ectodomain CAR-T cells compared to HIV-specific TCR-T cells. However, the underlying mechanism remains to be elucidated. Here, we show that CAR T cells can kill target at a faster pace because it is under constant degranulation. Our research provides a mechanistic explanation for the superiority of CD4 CAR-T cells in vitro and new insights into the strategy to use CD4 CAR-T cells as a therapy.

Mucosal Immunology

P23 Analysis of the mechanisms that sustain regulatory T cells in the small intestine

Elisa Cruz and Terri Laufer

Regulatory T cells (Tregs) are important for maintaining intestinal homeostasis. In steady state, the pool of small intestine lamina propria (siLP) Tregs includes two subpopulations: peripheral Tregs that are microbiota specific and express RORgt and a Helios+ subpopulation of natural Tregs that developed in the thymus. We previously showed that Helios+ thymic Tregs filled the siLP of K14 transgenic mice lacking peripheral TCR-MHCII and the differentiation of RORgt+ peripheral Tregs. We now ask how the Helios+ and RORgt+ siLP Treg subpopulations are maintained in the siLP with a specific focus on the MHCII-dependence of costimulatory signals. We used blocking reagents to examine the differential requirements for CD28, CTLA4, and ICOS in the proliferation, maintenance, and survival of siLP Tregs. We found that the relative proportion of RORgt+ and Helios+ Tregs in siLP is differentially modulated by blockade of ICOS or CD28. Helios+ Tregs rely only on CD28 and require neither ICOS nor MHCII signaling for their expansion; MHCII, CD28, and ICOS signaling contribute to RORgt+ Treg maintenance. We find that the composition of the Treg pool is dynamic; both subpopulations expand to compensate for the loss of the other to maintain total siLP Treg numbers. Together, our data highlight the intricate signaling network that regulates the intestinal Treg homeostasis.

Systems Immunology and Genomics

P24 Proteogenomic immune signatures delineate the landscape of pediatric acquired demyelinating syndromes

Diego A Espinoza, Ina Mexhitaj, Jacqueline Smiler, Fernanda Mafra, Renata Pellegrino Da Silva, Rui Li, Brenda Banwell and Amit Bar-Or

Approximately 20-30% of children presenting with acquired inflammatory demyelinating syndromes (ADS) have multiple sclerosis (MS). Another 30% harbor serum antibodies against myelin oligodendrocyte glycoprotein and are referred to as having MOG-associated disease (MOGAD). While MS and MOGAD can have similar features, differences in response to immune therapies point to distinct underlying immune mechanisms.
To assess potentially distinct immune mechanisms underlying MS and MOGAD, we applied proteogenomics to high quality cryopreserved peripheral blood mononuclear cells collected from patients with ADS prior to institution of immune therapy, as well as from healthy controls. CITE-Seq profiling was applied to a total of 92,716 single cells with equal contribution from 24 children (6 healthy donors; 6 with ADS but neither MS or MOGAD; 6 with MOGAD; and 6 with MS, ascertained with long-term follow-up).
Analysis revealed a pan-ADS enrichment of atypical (CD11c+) B cells compared to healthy controls. Children with MS were distinguished from children with MOGAD by MS-specific enrichments of checkpoint-molecule (TIGIT and CD137)-expressing CD8 memory T cells, STAT4+ Th1 CD4 memory T cells and CD56dim NK cells.
Overall, our study identifies distinct features of circulating cellular immune profiles that may serve to distinguish children with MS and MOGAD and provides novel insights into early immune mechanisms that may be involved in each of these conditions.

P25 Antagonism of De-repressed Endogenous Retroviruses MMTV and MMVL30 Across CD8+ T Cell States

Max Klapholz, Jingya Qiu, Josephine Giles, Jean-Christophe Beltra, Yinghui Jane Huang, Andy J. Minn and E. John Wherry

Endogenous retroviruses (ERVs) are largely inactive at homeostasis, and several epigenetic mechanisms function to repress ERVs to maintain genomic integrity. Recent work has demonstrated that ERV-derived elements are frequently de-repressed in the context of chronic inflammation, as in chronic infection, autoimmunity, and cancer. During chronic inflammation, persistent antigen- and cytokine-induced signaling contribute to CD8+ T cells acquiring a state of dysfunction or “exhaustion”, which impairs their ability to adequately clear pathogens or tumors and is characterized by a distinct transcriptional and epigenetic profile. Given that ERVs are more abundant in mammalian genomes than canonical protein-coding genes, we reasoned that ERV de-repression may contribute to T cell state-specific programs. We evaluated the single-cell expression of ERVs and other transposable elements in a recent scRNA-seq dataset (Giles et al bioRxiv 2022) of longitudinally profiled antigen-specific CD8+ T cells during acute or chronic LCMV infection, capturing naïve, effector, memory, and exhausted CD8+ T cells. Expression of transposable elements alone is sufficient to distinguish major CD8+ T cell states in UMAP reduced-dimensional space. Specifically, MMVL30 and RLTR6 were highly expressed in naïve and memory cells, less in effector cells, and the least in terminally exhausted cells. Oppositely, MMTV (murine mammary tumor virus) and RLTR3 expression was mostly restricted to terminally differentiated exhausted cells. We observed a similar pattern of MMVL30/RLTR6 and MMTV/RLTR3 expression in the bulk RNA-seq profiles of FACS-sorted subsets of exhausted CD8+ T cells representing a continuum of differentiation (Beltra et al Immunity 2020). Further, although both full-length and truncated MMVL30/RLTR6 loci drive variance in MMVL30/RLTR6 expression, only 2 full-length MMTV/RLTR3 loci drive the differential expression of MMTV/RLTR3. Together these analyses suggest that MMVL30/RLTR6 expression is abundant

in CD8 T cells prior to terminal differentiation, while MMTV/RLTR3 is mostly de-repressed in terminally exhausted cells.
shRNA-mediated knockdown of MMTV simultaneously increased MMVL30 expression in vitro. This effect was reversed in cells treated with dexamethasone, a synthetic glucocorticoid known to potently induce MMTV transcription. All together, these data suggest that MMTV is de-repressed in terminally exhausted CD8+ T cells and antagonizes MMVL30, an ERV expressed in CD8+ T cells with greater cytotoxic potential.
Further work is required to substantiate the impact of perturbing MMTV in vivo, defining the mechanisms whereby MMTV and MMVL30 may regulate CD8+ T cell biology, the potential human impact of MMTV de-repression in mice, and whether human ERVs might similarly modulate CD8+ T cell states.

P26 Uncovering Nutrient-Sensitive Modes of Gene Regulation in T Cells

Michael Scaglione, Kelly Rome, Crystal Conn and Will Bailis

T lymphocytes travel through a variety of metabolic environments - from central lymphoid structures and circulation into inflamed, hypoxic, metabolically disturbed peripheral sites - to exert their functions. Moreover, T cells have been shown to be sensitive to their nutrient environment and alterations in nutrient uptake or utilization have potent effects on T cell fate and function. However, the mechanisms directly linking changes in metabolic environment to control of T cell functional gene expression are incompletely understood. I have found that many functional genes are differentially sensitive to acute levels of glucose, glutamine, and other amino acids, with each nutrient controlling shared and unique modules of transcripts and displaying unique kinetics of regulation. These nutrients impact a wide variety of genes associated with T cell function (including cytokines, transcription factors, and metabolic enzymes). Notably, some changes I have observed occur rapidly, with some transcript-level changes detectable within 30 minutes. These findings, together with existing literature, suggests that there are discrete, nutrient-specific gene networks and gene regulation mechanisms. I hypothesize that T cells directly sense levels of these nutrients or their downstream metabolic products and utilize nutrient-sensitive mechanisms of transcriptional, post-transcriptional, and translational gene regulation to acutely govern gene expression in response to changes in nutrient environment. Using polysome profiling, we have mapped the nutrient-specific alterations in the cytotoxic T cell transcriptome and translatome under acute nutrient deprivation and aim to elucidate both the molecular features that grant a given gene product sensitivity to nutrient-mediated regulation as well as the cellular “sensors” responsible for enforcing changes in gene expression. Better understanding of these mechanisms will not only provide basic insight into how T cell function is regulated by metabolism but may also be utilized to promote T cell function in metabolically challenging sites such as tumors during endogenous anti-cancer immune responses or adoptive cancer immunotherapies.

P27 Utility of Biomedical Knowledge Base Integration for Advancing Immune Health Research

Van Truong, Joseph D. Romano, Scott M. Dudek, Allison R. Greenplate, E. John Wherry and Marylyn D. Ritchie

The human immune system is composed of many cell types and molecular components, with vast variation across individuals. By nature, biological information is extremely complex and difficult to visualize in its entirety. Despite decades of advancements, methods development and applications of artificial intelligence (AI) in immune health research remain rudimentary. Thus, biomedical informatics is well-suited to unify diverse knowledge types spanning the genome, transcriptome, proteome, biological pathways, and disease association in a machine-readable manner. To bridge this gap, we unite open access biomedical databases into a central knowledge base (KB) and discuss the development of methods capable of utilizing the underlying

network structure to direct predictive modeling for the dissection of complex immunological diseases.
Here, we employ the Neo4j graph database platform and Cypher query language, which are well-suited for storing and analyzing semantically meaningful relationships within biological and immunological information networks. First, we integrated several sources of curated biological knowledge from Hetionet, ComptoxAI, Reactome, Pathway Commons, and Library of Knowledge Integration (LOKI), and incorporated additional immunology-related entities: ImmGen, ImmunoGlobe, and immune-associated diseases queried from DisGeNet. In the graph, the nodes correspond to individual entities in the source databases while edges represent the mapped relationships between nodal entities. We are continually developing the knowledge infrastructure to provide additional data-types and expanding its utility and functionality. Next, we aim to develop graph data science approaches for knowledge discovery such as heterogeneous graph machine learning (ML) models for link prediction. We will also explore regularization and feature selection strategies to improve ML applications on highly connected, complex information. Coupled together, we believe our work will provide a path forward to explore data beyond single data-types and embrace a meta-dimensional framework for modeling strategies and applications in Immune Health.

Transplantation

P28 Trafficking and Persistence of Alloantigen-Specific Chimeric Antigen Receptor Regulatory T cells in Cynomolgus macaque

Gavin Ellis, Kimberly E. Coker, Delaine W. Winn, Mosha Z. Deng, Divanshu Shukla, Vijay Bhoj, Michael C. Milone, Wei Wang, Chengyang Liu, Ali Naji, Raimon Duran-Struuck and James L. Riley

Adoptive transfer of chimeric antigen receptor regulatory T cells (CAR Tregs) is a promising way to prevent allograft loss without the morbidity associated with current therapies. Non-human primates (NHPs) are a clinically relevant model to develop transplant regimens, but manufacturing and engraftment of NHP CAR Tregs has not been demonstrated yet. Here, we describe a culture system that massively expands CAR Tregs specific for the Bw6 alloantigen. In vitro, these Tregs suppress in an antigen-specific manner without pro-inflammatory cytokine secretion or cytotoxicity. In vivo, Bw6 specific CAR Tregs preferentially traffic to and persist in bone marrow for at least 1 month. Following transplant of allogeneic Bw6+ islets and autologous CAR Tregs into the bone marrow of diabetic recipients, CAR Tregs traffic to the site of islet transplantation and maintain a phenotype of suppressive Tregs. Our results establish a framework for the optimization of CAR Treg therapy in NHP disease models.

P29 Leveraging CAR T cells to Achieve Desensitization and Enable Transplantation

Zheng Zhang, Caroline Markmann, Ming Yu, Susan Rostami, Wei Wang, Trini Ochoa, Kalpana Parvathaneni, Xiaoming Xu, John Scholler, Qian Zhang, Avery Posey, David Allman, Michael Milone, Valder Arruda, Ben Samelson Jones, Ali Naji, Vijay Bhoj

Pre-existing allo-antibodies (allo-Abs), that preclude transplant due to the risk of hyperacute rejection, lead to prolonged wait times and high mortality rates. Current desensitization approaches are ineffective as they do not adequately deplete allo-specific B cells and plasma cells (PCs). We hypothesize that stringent depletion of these cells is required to eliminate pre-existing allo-Abs. We leverage the exquisite ability of CAR T cells to eliminate target cells to desensitize transplant candidates. We constructed CARs targeting murine CD19 or BCMA, which cover the entire B cell-PC continuum. We first evaluated the function of CAR T cells against B cells and PCs in vitro. C57BL/6 mice were sensitized with BALB/c skin grafts. After skin rejection, sensitized mice received total body irradiation followed by treatment with either control

T cells, CART-19 T cells, or a combination of CART-19 and CART-BCMA T cells (combo-CART). Allo-Abs, total Ig, and B cells were measured over 13 weeks. Functional desensitization was then assessed by induction of diabetes followed by BALB/c-derived islet cell transplant and glucose was measured to assess graft survival. CD19- and BCMA-targeted CARs effectively depleted primary B cells and PCs in vitro and in vivo. Control and CART-19 T cells were ineffective at desensitizing mice, but combo-CART treatment resulted in significant decrease of allo-Abs. Islet cell grafts succumbed to hyperacute rejection in 80% of control and CART-19 treated mice. However, combo-CART treatment resulted in prolonged graft survival in all mice (mean 35 days, range 16-60). Thus, CAR T cells targeting B cell and PC antigens represent a promising approach to desensitization and could enable life-saving transplantation.

P30 The role of the Type III Secretion System in MHCII antigen presentation of Salmonella enterica epitopes.

Kathleen Krauss, Chaitali Bhadiadra, Michael Hogan, and Laurence Eisenlohr

Salmonella enterica is a bacterial pathogen with an ingenious niche: a phagosome reprogrammed via bacterial effector proteins secreted into the host cytosol. CD4 T cells form the backbone of an efficient response to *S enterica* infection by activating infected macrophages. However, the role of the Salmonella containing vacuole on formation of CD4 T cell responses remains relatively unexplored. The Eisenlohr lab has performed a screen of predicted Salmonella CD4 epitopes in mice and found that CD4 responses are highly skewed toward the secreted effector proteins at the expense of other bacterial proteins sequestered in the vacuole. This suggests that secreted effectors in the cytosol of infected APCs are an abundant source of peptide to present to CD4 T cells, running counter to the classical paradigm of CD4 antigens originating from material outside the presenting cell. Understanding this skew could not only improve our understanding of the immune response to *S enterica* but could also illuminate a novel pathway for antigen presentation in bacterial infections. I plan to (1) investigate the role of T3SS in maintaining the skew towards effector proteins in presentation, (2) explore the subcellular origin of Salmonella antigen presented to T cells by testing proteasome dependence, (3) test the protection of CD4 T cells responsive to secreted effector antigen versus antigen sequestered in the vacuole. To answer these questions, I will use an mRNA vaccine system pioneered by the Eisenlohr lab to generate epitope-specific CD4 T cell responses. This line of investigation could yield insights into both vaccine design and a novel means of antigen presentation.

Acknowledgements: Thank you for all our invited speakers for participating in this event. A special thanks to the IFI administrative staff for their tireless efforts.

Front Cover Image Description and Credit: *(right image) This is an image of the Cryptosporidium parvum expressing an epitope tagged transport protein which localizes at the interface between the parasite and the host cell (seen as a red ring). Blue = Hoechst, Green = Cryptosporidium marker, red = transport protein, Bethan (Hunter Lab); (left image) • Section of Cryptosporidium infected intestine stained for with EdU to track proliferating cell migration. Blue = Hoechst, green = Phalloidin, red= EdU., Bethan (Hunter Lab)*

Notes