Stanford Immunology Annual Scientific Conference

November 10 – 12, 2023

Stanford MEDICINE
School of Medicine
Immunology

AGENDA & ABSTRACTS

ASILOMAR CONFERENCE GROUNDS
800 ASILOMAR AVE, PACIFIC GROVE, CA 93950
https://stan.md/47gMUW5

Stanford Immunology Quick Facts

Program launch

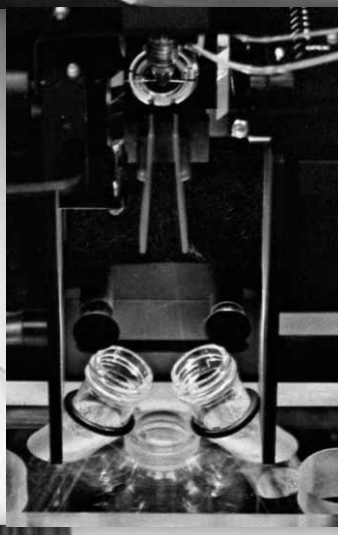
- 1988

Directors:

- Irving Weissman (Pathology/Developmental Biology)
- Hugh McDevitt (Microbiology and Immunology/Medicine)
- Larry Steinman (Neurology)
- Pat Jones (Biology)
- Olivia Martinez (Surgery, as of July 2018)

Director of the Institute for Immunology, Transplantation, and Infection (ITI)

- Established 2004
- Mark Davis (Microbiology and Immunology)
- Bali Pulendran (Microbiology and Immunology, incoming 2024)



Images from Jones, P.P., Herzenberg, L.A. The early history of Stanford Immunology. *Immunol Res* **58**, 164–178 (2014). https://doi.org/10.1007/s12026-014-8518-z

Updated 11.06.23

WELCOME

Stanford MEDICINE Immunology
School of Medicine

Welcome to the November 2023 Stanford Immunology Program's Annual Conference at Asilomar!

We are pleased to present what promises to be an exciting retreat, loaded with presentations by faculty, staff, postdocs, and graduate students from across the Stanford Immunology community. Talks and poster session will be held in Merrill Hall. Talks are divided into sessions of common theme with innovative research that crosses traditional boundaries at Stanford University School of Medicine. You won't want to miss a single talk! There are plenty of breaks to re-caffeinate, rehydrate, sugar up, and network. Faculty talks are 15 minutes, and graduate student, postdoc and staff talks are 10 minutes each. We will be enforcing the time limits to provide at least 5 minutes for questions, related insights, and to catalyze discussions throughout the weekend.

KEYNOTE SPEAKERS

We are honored to have two keynote speakers:

Mark Davis, PhD
Stanford University

Ellen Robey, PhD
UC Berkeley

Mark M. Davis, PhD is the Director of the Stanford Institute for Immunology, Transplantation and Infection (ITI), a Professor of Microbiology and Immunology, and a Howard Hughes Medical Institute Investigator. He received a B.A. from Johns Hopkins University and a Ph.D. from the California Institute of Technology. He later was a postdoctoral fellow and staff fellow at the Laboratory of Immunology at NIH and later became a faculty member in the Department of Microbiology and Immunology at Stanford University School of Medicine, where he remains today. Dr. Davis is well known for identifying many of the T-cell receptor genes, which are responsible for the ability of these cells to recognize a diverse repertoire of antigens. Other work in his laboratory pioneered studies of the biochemistry, genetics and cell biology of these molecules and T lymphocytes generally, which play a key role in orchestrating immune responses. His current research focuses on obtaining a "systems level" understanding of the human immune system. This has involved the steady state and vaccine responses of old and young subjects, as well as a recent study of twins, which concluded the variation in most immune system parameters is not driven by inherited variation, but rather by environmental factors.

Ellen Robey is currently a Professor of Immunology and Molecular Medicine in the Molecular & Cell Biology Department at the University of California, Berkeley. Robey graduated Phi Beta Kappa with a BA in Biology from the University of Virginia in 1981. She received a PhD in Biochemistry from UC Berkeley in 1986, and did her postdoctoral research at Columbia University, examining the role of CD4 and CD8 in T cell fate decisions. She has been a professor at Berkeley since 1992, and was promoted to full professor in 2004. She was named a Miller Professor in 2007 and an AAI Distinguished Fellow in 2022.

Research in the Robey lab is focused on understanding the development and function of the mammalian immune system with a focus on in vivo mouse models. Her lab has been at the forefront of applying advanced microscopy to directly visualize the immune system in action. Most recently she and her collaborators have applied multiomic approaches to dissect T cell fate determination in the thymus.

T cell fate determination in the thymus: a multi-omic approach

The development of $CD4^+$ T cells and $CD8^+$ T cells in the thymus is critical to adaptive immunity and is widely studied as a model of lineage commitment. Recognition of self-MHCI or MHCII by the T cell antigen receptor (TCR) determines the $CD8^+$ T cell or $CD4^+$ T cell lineage choice, respectively, but how distinct TCR signals drive transcriptional programs of lineage commitment remains largely unknown. Here we applied CITE-seq to measure RNA and surface proteins in thymocytes from wild-type and T cell lineage-restricted mice to generate a comprehensive timeline of cell states for each T cell lineage. These analyses identified a sequential process whereby all thymocytes initiate $CD4^+$ T cell lineage differentiation during a first wave of TCR signaling, followed by a second TCR signaling wave that coincides with $CD8^+$ T cell lineage specification. CITE-seq and pharmaceutical inhibition experiments implicated a TCR-calcineurin-NFAT-GATA3 axis in driving the $CD4^+$ T cell fate. Our data provide a resource for understanding cell fate decisions and implicate a sequential selection process in guiding lineage choice.

Our alumni panelists include:

Michael N. Alonso, PhD, Vice President, Immunology & Pharmacology at Bolt Biotherapeutics

Michael Alonso, Ph.D., Vice President, Immunology & Pharmacology, is scientific co-founder at Bolt Biotherapeutics and co-inventor of Bolt's proprietary ISAC platform. Dr. Alonso is passionate about translating scientific discoveries into innovative therapies that ensure cancer patients become cancer survivors. Dr. Alonso earned his Ph.D. in Immunology from Stanford University School of Medicine in 2011 where he identified a positive feedback loop between CD4+ T cells and monocytes that results in the formation of specialized dendritic cell subsets as a member of Dr. Engelman's laboratory. Dr. Alonso earned his Certificate in Entrepreneurship from the Stanford Graduate School of Business and graduated with distinction from the University of Illinois at Urbana-Champaign.

Zina Good, PhD, Instructor
Stanford Institutes of Medicine

Zinaida Good, PhD is an instructor working at the interface between systems biology and cancer immunotherapy at Stanford University. Dr. Good's research with Profs. Crystal L. Mackall and Sylvia K. Plevritis is focused on investigating why chimeric antigen receptor (CAR) T cell immunotherapies succeed or fail in patients, and she has recently identified CAR T regulatory cells as a correlate of progression following CD19-CAR therapy for large B cell lymphoma. Dr. Good earned her Ph.D. in Computational & Systems Immunology from Stanford University, where she trained with Profs. Garry P. Nolan and Sean C. Bendall and defined methods to build and leverage lymphocyte differentiation trajectories in health and cancer. Her background is in experimental immunology and oncology and combines 2 years of experience working in

Discovery Oncology at Genentech with B.S. and M.S. degrees in Microbiology & Immunology from the University of British Columbia, where she investigated the mechanisms of T cell memory with Prof. Michael R. Gold. Dr. Good's work includes 4 first-author papers (Nature Medicine 2018 & 2022, Nature Biotechnology 2019, Trends in Immunology 2019), 12 co-authored papers (including Nature 2019 & 2022, Science 2021, Nature Methods 2016 & 2022), and 2 patent applications. Her academic potential has been recognized by prestigious postdoctoral fellowships (2018 Parker Institute for Cancer Immunotherapy Scholar, 2020 Stanford Cancer Institute Fellow), a career development award (2023 Parker Institute for Cancer Immunotherapy Bridge Fellow), and she has been named an Arthur and Sandra Irving Cancer Immunology Fellow in 2022. Dr. Good is preparing to launch an independent research program with a long-term goal to understand and enhance engineered cellular immunotherapies for patients with cancer.

Murad Mamedov, PhD, Postdoctoral Fellow
Gladstone-UCSF Institute of Genomic Immunology

Murad Mamedov is a postdoctoral fellow at the Gladstone-UCSF Institute of Genomic Immunology in Alex Marson's laboratory. His work is at the intersection of gamma-delta T cells, cancer, functional genomics, and CRISPR editing. Specifically, Murad studies the immune system's capacity for sensing cellular stress through the lens of gamma-delta T cells. His research was supported by the Michelson Prize for Human Immunology and the Cancer Research Institute (CRI) Irvington Postdoctoral Fellowship. Prior to his postdoctoral work, Murad completed an undergraduate B.S. degree in Biology and Philosophy at Georgetown University, and then completed an immunology Ph.D. in Mark Davis's laboratory at Stanford. During his Ph.D. work, Murad studied the role of gamma-delta T cells and gamma-delta T cell receptors during malaria.

ACTIVITIES

Game Show Friday
On Friday night, after the keynote, we will have the traditional game show featuring our faculty and Second Year Immunology students.

Poster Session Saturday
The poster session will be on Saturday night at 8:30-11:00 pm, along with a reception. A Best Poster Prize will be given to two graduate students and two postdoctoral fellows.

Poster Ads will run continuously throughout the retreat. The intent of these Poster Ads is to draw as many people as possible to the posters. Voting for Best Poster will be done democratically – everyone votes! Faculty are encouraged to visit each poster.

Birukova Midnight Swim
Take a plunge in the ocean on Friday night! In honor of Maria Birukova, a former School of Medicine graduate student, students, postdocs, and faculty can continue the tradition of the midnight swim on Saturday.

CDIII Updates Saturday
The CDIII (Community, Diversity and Inclusion In Immunology) committee aims to promote a culture of diversity, equity and inclusion in the Immunology Program through the identification and recommendation of long- lasting creative solutions that embrace anti- racism principles and eliminate systemic bias. They will provide updates on activities and programs happening throughout the year.

Faculty-First Years only Research Blitz
On Saturday, faculty wishing to recruit rotation students to their labs may participate in a research blitz with first year students. Each faculty member will have the opportunity to talk about their research in several minutes and converse with a First Year student. Once the allotted few minutes are up, the faculty member will meet the next first year student.

Career Panel Sunday
Graduate students and postdoc fellows have the chance to meet Stanford Immunology alumni talk about academic careers, careers in the industry, and real-life experiences.

Take a walk
Take a quick stroll to Asilomar State Beach to breathe in some fresh air. Meet at Crocker Hall after you grab some coffee and breakfast.

Saturday, November 11th
8:15-8:45 AM

Let's decorate
What do you get when you combine the beach and a gingerbread house? Team with up to 4 people and get creative with Gingerbread Beach House decorating. *Vote for your favorite decorated house!*

Saturday, November 11th
3:00-4:00 PM at Merrill Hall

The Annual Scientific Conference is one of the highlights of the academic year for our Stanford Immunology community – we thank you for participating in this marvelous and enriching experience.

November 2023 Immunology Conference Committee
Thank you to the conference planning committee and your dedication on making the event a success.

Conference Directors: Drs. Jennifer Bando and Nathan Reticker-Flynn
Social Chairs: Second Year Class
Conference Committee: Dr. Olivia Martinez, Torye Nguyen, and Lina Hansen
Staff Support: Lynn Galicia, Cindy Limb, and Rita Robinson
Technical Support: Candace Liu

PROGRAM AT A GLANCE

Stanford MEDICINE Immunology
School of Medicine

Times, speakers, and topics may change without notice.

Friday, November 10		
12:45-1:00 PM	**Conference Check-in**	Merrill Hall
1:00-1:10 PM	**Welcome and Introductions**	Merrill Hall
1:10-2:00 PM	**Session 1**	Merrill Hall
1:10-1:30 PM	**Tobias Lanz, MD**, Assistant Professor of Immunology & Rheumatology, *Identifying pathogenic viral and myelin antigens in multiple sclerosis*	Merrill Hall
1:30-1:45 PM	**David Lee**, Research Associate, Kirane Lab, *Biomarker analysis of intralesional neoadjuvant TVEC for high-risk Stage II melanoma: A Phase II Clinical Trial*	Merrill Hall
1:45-2:00 PM	**Camilo Espinosa Bernal**, Immunology Graduate Student, Aghaeepour Lab, *Multiomics characterization of acute child illness and post-discharge mortality in Africa and South Asia*	Merrill Hall
2:00-2:45 PM	**Session 2**	Merrill Hall
2:00-2:15 PM	**Savannah Lewis**, Microbiology and Immunology Graduate Student, Jagannathan Lab, *Evaluating the impact of natural killer cell phenotype, malaria diversity and transmission, and erythrocyte polymorphisms on antibody-dependent cellular cytotoxicity*	Merrill Hall
2:15-2:30 PM	**Ayan Mondal, PhD**, Postdoc, Mellins Lab, *Matrix Metalloprotease 9 (MMP9) induction in brain endothelium may lead to blood-brain barrier dysfunction in Pediatric Acute Neuropsychiatric Disorder (PANS)*	Merrill Hall
2:30-2:45 PM	**Neetu Saini, PhD**, Postoc, Bacchetta Lab, *Generation of gut-specific-engineered Treg-like cells (GI-CD4LVFOXP3) for pediatric Crohn's disease treatment*	Merrill Hall
2:45-3:00 PM	**Break**	Merrill Hall
3:00-3:50 PM	**Session 3**	Merrill Hall
3:00-3:20 PM	**Eric Meffre, PhD**, Professor of Immunology & Rheumatology, *TLR9 ligand sequestration by chemokine CXCL4 abrogates central B cell tolerance*	Merrill Hall
3:20-3:35 PM	**Qiwen Deng, PhD**, Postdoc, Wernig Lab, *Decoding the spatial transcriptomic landscape of diabetic nephropathy*	Merrill Hall
3:35-3:50 PM	**Rebeca Arroyo Hornero, PhD**, Postdoc, Idoyaga Lab, *A fraction of human plasmacytoid dendritic cells transdifferentiate into conventional dendritic cell type 2 following activation*	Merrill Hall
3:50-4:50 PM	**Session 4**	Merrill Hall
3:50-4:05 PM	**Grayson Rodriguez**, Immunology Graduate Student, Garcia Lab, *Rebalancing STAT signaling with non-natural cytokine receptor combinations to modulate immune cell functionality*	Merrill Hall

4:05-4:20 PM	**Vishnu Shankar**, Immunology Graduate Student, Davis and Mischel Labs, *Oxidative Stress is a shared characteristic of ME-CFS and Long COVID*	Merrill Hall
4:20-4:35 PM	**Alex Muselman**, Immunology Graduate Student, Engleman Lab, *Intracranial infection with gammaherpesvirus-68 primes microglia to a hyperinflammatory state and drives atypical EAE*	Merrill Hall
4:35 PM	**Lodging Check in**	Front Desk
6:00-7:00 PM	**Dinner**	Crocker Hall
7:05-8:05 PM	**Keynote: Mark Davis, PhD**, Director of the Stanford Institute for Immunology, Transplantation and Infection (ITI), Professor of Microbiology and Immunology	Merrill Hall
8:05 PM	**Break**	Merrill Hall
8:30 PM	**Game Show & Reception**	Merrill Hall

Saturday, November 11		
7:30-9:00 AM	**Breakfast**	Crocker Hall
8:15-8:45 AM	**Morning Walk to the Beach**	Asilomar State Beach
9:00-9:50 AM	**Session 5**	Merrill Hall
9:00-9:20 AM	**David Lewis, MD,** Professor of Pediatrics - Immunology and Allergy, *Using CRISPR-edited induced pluripotent stem cells (iPSCs) to define mechanisms of disease in Schimke Immuno-Osseous Dysplasia (SIOD), a monogenic primary immunodeficiency and complex disease*	Merrill Hall
9:20-9:35 AM	**John Hickey, PhD**, Postdoc, Nolan Lab, *Associations of immune hubs in metaplastic progression to adenocarcinoma revealed by high-speed multiomic spatial phenotyping of FFPE human samples*	Merrill Hall
9:35-9:50 AM	**Kalani Ratnasiri**, Immunology Graduate Student, Blish and Khatri Labs, *Conserved monocyte responses to acute RNA viruses*	Merrill Hall
9:50-10:05 AM	**Break**	Merrill Hall
10:05-10:50 AM	**Session 6**	Merrill Hall
10:05-10:20 AM	**Dongeon Kim, PhD**, Postdoc, Nicholls Lab, *Abnormal lymphatic S1P signaling aggravates lymphatic dysfunction and tissue inflammation*	Merrill Hall
10:20-10:35 AM	**Brenda Velasco**, Immunology Graduate Student, Shizuru Lab, *The effects of anti-CD117 monoclonal antibody on the bone marrow microenvironment*	Merrill Hall
10:35-10:50 AM	**Simon Borna, PhD**, Postdoc, Bacchetta Lab, *Analyses of Treg plasticity and TCR repertoire autoreactivity in patients with FOXP3 mutation*	Merrill Hall
10:50-11:00 AM	**Awards Presentation**	Merrill Hall
11:00-11:45 AM	**CDIII Updates**	Merrill Hall
11:45 AM-12:00 PM	**Group Pictures**	Merrill Hall
12:00-1:00 PM	**Lunch**	Merrill Hall

12:15-1:15 PM	**Faculty Meeting**	Merrill Hall
1:30-3:00 PM	**Research Blitz with First Years and Faculty**	Merrill Hall
3:00-4:00 PM	**Gingerbread Beach House Decorating**	Merrill Hall
6:00-7:00 PM	**Dinner**	Crocker Hall
7:05-8:05 PM	**Keynote: Ellen Robey, PhD,** Professor of Immunology and Pathogenesis, University of CA, Berkley, *T cell fate determination in the thymus: a multi-omic approach*	Merrill Hall
8:05-8:30 PM	**Break**	Merrill Hall
8:30-11:00 PM	**Poster Session**	Merrill Hall
11:00 PM-12:00 AM	**Bonfire**	BBQ Area
12:00 AM	**Birukova Midnight Swimming Club**	Ocean

Sunday, November 12		
7:30-9:00 AM	**Breakfast**	Crocker Hall
9:00-9:50 AM	**Session 7**	Merrill Hall
9:00-9:20 AM	**Derick Okwan, MD, PhD**, Assistant Professor of Pathology, *Role of neutrophils in brown adipose thermogenesis*	Merrill Hall
9:20-9:35 AM	**Adonis Rubio**, Immunology Graduate Student, Barnes Lab, *Engineering bispecific antibodies that recognize the SARS-CoV-2 Spike glycoprotein N-terminal and receptor binding domains*	Merrill Hall
9:35-9:50 AM	**Ana Jimena Pavlovitch-Bedzyk**, Immunology Graduate Student, Davis Lab, *Immune competent air liquid interface skin organoids reveal monkeypox dynamics*	Merrill Hall
9:50-10:05 AM	**Break**	Merrill Hall
10:05-11:00 AM	**Career Panel: Michael Alonso, PhD**, Vice President of Immunology & Pharmacology at Bolt Biotherapeutics, **Zina Good, PhD**, Instructor at Stanford Institutes of Medicine, **Murad Mamedov, PhD**, Postdoctoral Fellow at Gladstone-UCSF Institute of Genomic Immunology	Merrill Hall
11:00-11:15 AM	**Break**	Merrill Hall
11:15 AM-12:15 PM	**Session 8**	Merrill Hall
11:15-11:30 AM	**Surbhi Sharma, PhD**, Postdoc, Mellins Lab, *Anakinra, an IL-1 inhibitor, alters peptide interactions with HLA-DR15, a risk haplotype of sJIA/DRESS.*	Merrill Hall
11:30-11:45 AM	**Hayley Raquer**, Immunology Graduate Student, Idoyaga Lab, *Ontogeny impacts Langerhans cell functional properties*	Merrill Hall
11:45 AM-12:00 PM	**Cameron Bader, PhD**, Postdoc, Meyer Lab, *Single CD4 T cell phenotypic structure and oligoclonal polarization favor FOXP3 and HELIOS over IFNG and proliferative genes in Orca T patients without GVHD*	Merrill Hall
12:00-12:15 PM	**Noor Hussein, PhD**, Postdoc, Mellins Lab, *Regulatory T cells subsets in Pediatrics Acute Onset Neuropsychiatric Syndrome (PANS)*	Merrill Hall
12:15-12:20 PM	**General Announcements**	Merrill Hall
12:20-1:00 PM	**Lunch and Checkout at Asilomar**	Crocker Hall

AWARDS

Stanford Immunology recognizes the extraordinary professional achievements and career promise of its graduate students. All awardees receive a certificate and $1,000 honorarium. Awardees to be announced.

The McDevitt Award recognizes excellence in a PhD student's doctoral dissertation research. The PhD candidate shall be chosen from a group of candidates who have defended and submitted their thesis to the Registrar in a given academic year.

Read more about **Hugh McDevitt, MD**.

The Jones Award recognizes a current PhD student for excellence in their instructional roles in the classroom and/or the laboratory.

More info on **Pat Jones, PhD**

The Service Award recognizes a current PhD student who has repeatedly demonstrated an exceptional commitment towards service.

Friday, November 10, 2023
Merrill Hall
Session 1

1:10-1:30 PM	**Tobias Lanz, MD**, Assistant Professor of Immunology & Rheumatology, *Identifying pathogenic viral and myelin antigens in multiple sclerosis*
1:30-1:45 PM	**David Lee**, Research Associate, Kirane Lab, *Biomarker analysis of intralesional neoadjuvant TVEC for high-risk Stage II melanoma: A Phase II Clinical Trial*
1:45-2:00 PM	**Camilo Espinosa Bernal**, Immunology Graduate Student, Aghaeepour Lab, *Multiomics characterization of acute child illness and post-discharge mortality in Africa and South Asia*

Tobias Lanz, PhD, Assistant Professor
Department of Immunology & Rheumatology
Identifying pathogenic viral and myelin antigens in multiple sclerosis

Multiple sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system (CNS), in which aberrantly activated B and T cells attack and destroy the myelin sheath in the CNS. B cell depletion is a highly effective therapy for the disease. Despite intense research, the specific B and T cell antigens have not yet been identified. We sequenced the single cell B cell repertoire in the cerebro-spinal fluid (CSF) of MS patients, and tested individual B cell receptors (BCRs) / antibodies for their reactivity to viral and myelin antigens. We identified a BCR that binds with high affinity to the Epstein Barr virus (EBV) transcription factor EBNA1 and cross-reacts to the glial cellular adhesion molecule GlialCAM. We could show that this autoreactive BCR derived from a naïve BCR that was only reactive to the viral antigen, and that somatic hypermutation enabled molecular mimicry. A posttranslational modification of the autoantigen GlialCAM is necessary for the BCR's high-affinity binding. In addition, we identified a BCR/autoantibody that binds to a conformational epitope of the major myelin protein proteolipid protein (PLP). PLP is a highly conserved complex membrane protein, which accounts for approximately 50% of the myelin protein mass. We can show that our newly identified anti-PLP antibody is highly pathogenic in the mouse model of MS. We assess anti-PLP reactivity as a potential biomarker for MS diagnosis and develop antigen-specific strategies for the treatment of the disease.

David Lee, Research Associate
Advisor: Amanda Kirane
Department of Surgery - General Surgery

Biomarker analysis of intralesional neoadjuvant TVEC for high-risk Stage II melanoma: A Phase II Clinical Trial

In this study, we explored the use of an oncolytic virus, Talimogene Laherparpvec (TVEC), before standard-of-care surgery (SOS) in high-risk stage II melanoma. The use of immunotherapy is controversial in patients without nodal disease, fostering the need for a safe and efficacious therapeutic agent in this population. TVEC has shown promise within the neoadjuvant setting in advanced melanoma, with a 34% complete response rate however no biomarkers of response have consistently predicted patients likely to benefit from therapy. In our Phase 2 clinical trial (NCT04427306), six patients completed TVEC therapy without experiencing any adverse effects beyond grade I. Three patients had partial pathologic responses, one had a complete pathologic response, and one had a positive sentinel node. No complications after SOS or relapses were noted. Immune fluorescence resonance energy transfer (iFret) showed varying alterations in T cell PD-1 receptor engagement after intralesional TVEC administration. Statistically significant increases in iFret efficiency ($p=0.007$) correlated with favorable proinflammatory response and a decrease or no change from baseline corresponded with the inverse. Mass cytometry (CyTOF) profiling revealed a correlation between response and monocyte population shifts as opposed to variations in the total CD4+ and CD8+ cells; however, further analysis revealed unique divergences in T cell subset expansion, exhaustion, and NKT cell populations. The summation of our data defines changes in multiple immune components which can help identify patients who are likely to gain advantages from neoadjuvant intralesional therapy and imply that baseline innate immune factors play a significant role in influencing immunotherapy response.

Camilo Espinosa Bernal, Immunology Graduate Student
Advisor: Nima Aghaeepour
Department of Anesthesiology, Perioperative and Pain Medicine, and of Pediatrics - Neonatal and Developmental Medicine

Multiomics characterization of acute child illness and post-discharge mortality in Africa and South Asia

Infant mortality remains unacceptably high and overwhelmingly prevalent in low- and middle-income countries (LMICs). Children in LMICs often exhibit unstable health trajectories after hospitalization, characterized by some patients exhibiting incomplete recovery and heightened risks of readmission and post-discharge mortality. However, targeted interventions to reduce these risks remain elusive. This work employed multiomic profiling and multivariate modeling to investigate the conserved biological mechanisms of inpatient and post-discharge mortality in LMICs. A multinational cohort of 3101 acutely-ill children across six LMICs was enrolled at hospital admissions. Clinical, demographic, and socioeconomic data and plasma, serum, stool, and fecal swab samples were collected at

admissions and discharge. Samples from 1008 participants selected in a nested case-control fashion were further analyzed to generate plasma proteomics, serum metabolomics and lipidomics, stool metagenomics, and fecal pathogen data. A machine learning-based multiomics model for predicting mortality had high accuracy at admission (AUROC=0.85) and moderate accuracy at discharge (AUROC=0.74). Features associated with mortality included perturbations in immune, inflammatory, and metabolic proteins in plasma; increased serum levels of multiple metabolites and lipids; and dysbiosis of gut microbiota. Correlation analysis revealed a strong association (r=0.66) between signals of mortality at admissions and discharge. Further examination of patients with a discrepancy between the severity of their clinical presentation and their predicted multiomic mortality risk provided specific measures for data-driven patient risk stratification. These results clarify the relationship between the biological mechanisms of acute illness and post-discharge mortality and shed light on potential interventions to reduce infant mortality in LMICs.

Friday, November 10, 2023
Merrill Hall
Session 2

2:00-2:15 PM	**Savannah Lewis,** Microbiology and Immunology Graduate Student, Jagannathan Lab, *Evaluating the impact of natural killer cell phenotype, malaria diversity and transmission, and erythrocyte polymorphisms on antibody-dependent cellular cytotoxicity*
2:15-2:30 PM	**Ayan Mondal, PhD**, Postdoc, Mellins Lab, *Matrix Metalloprotease 9 (MMP9) induction in brain endothelium may lead to blood-brain barrier dysfunction in Pediatric Acute Neuropsychiatric Disorder (PANS)*
2:30-2:45 PM	**Neetu Saini, PhD**, Postoc, Bacchetta Lab, *Generation of gut-specific-engineered Treg-like cells (GI-CD4LVFOXP3) for pediatric Crohn's disease treatment*

Savannah Lewis, Microbiology & Immunology Graduate Student
Advisor: Pras Jagannathan
Department of Medicine - Infectious Diseases, and of Microbiology & Immunology
Evaluating the impact of natural killer cell phenotype, malaria diversity and transmission, and erythrocyte polymorphisms on

In recent years, the ability of natural killer (NK) cells to mediate antimalarial immunity through antibody-dependent cellular cytotoxicity (ADCC) has become increasingly appreciated. Our group recently reported that an atypical population of NK cells prevalent in malaria-exposed children has enhanced ADCC activity against opsonized infected erythrocytes superior to that of conventional NK cells. However, ADCC was only evaluated in wild-type erythrocytes infected with laboratory-adapted P. falciparum, despite the high prevalence of hemoglobin polymorphisms in endemic areas and antigenic diversity of field isolates. Furthermore, extensive cellular phenotyping to find features associated with enhanced cytotoxicity and malaria exposure have not been conducted. This work aims to characterize the impact of parasite diversity, erythrocyte polymorphism, NK cell phenotypes, and malaria exposure on the ability to mediate ADCC against infected erythrocytes. We have already found that HbAS erythrocytes and plasma from high-transmission areas enhance ADCC in comparison to HbAA erythrocytes and low-transmission plasma. Experiments in progress are characterizing NK cell phenotypic traits associated with ADCC capacity in malaria-exposed Ugandans in comparison to malaria-naive North Americans. Peripheral blood mononuclear cell (PBMC) samples from the PRISM (Program for Resistance, Immunology, Surveillance, and Modelling of Malaria) observational study cohort in Nagongera, Uganda and malaria-naive North Americans are stimulated with infected erythrocytes or pro-inflammatory cytokines analyzed for phenotypic markers and activation using multiparameter flow cytometry. These results will help improve the translatability of future research by creating experimental conditions that better reflect malaria-endemic settings.

Ayan Mondal, PhD, Postdoc
Advisor: Elizabeth Mellins
Department of Pediatrics - Human Gene Therapy

Matrix Metalloprotease 9 (MMP9) induction in brain endothelium may lead to blood-brain barrier

The blood-brain barrier (BBB) is formed by a monolayer of tightly sealed brain endothelial cells (BEC) and maintained by interactions between tight junction proteins [e.g., claudin 5, occludin, and zonula occludens 1 (ZO1)] and adherens junction proteins [e.g., VE Cadherin, β catenin]. Impaired BBB function is hypothesized to play the role in the development of PANS, a disorder characterized by neuroinflammation and microglial activation following leukocyte or autoantibody infiltration into the central nervous system from the circulation. To begin to test this hypothesis, we used a BEC monolayer as an in vitro BBB model. We measured BEC permeability and junctional integrity after exposure to plasma from PANS patients with active disease (acute and chronic), the same patients after clinical improvement, and age-sex-matched healthy controls. Plasma from active PANS at 1% volume significantly increased BEC permeability to 10 kDa dextran compared to healthy control plasma. Increased permeability was correlated with BEC structural damages, including disruption of the adherent junction protein VE-Cadherin and formation of actin stress fibers. In addition, we observed an induced expression of active MMP9 from BEC with plasma from active PANS (acute), which may cause BEC structural deformation and lead to dysfunction. Further research is underway to elucidate mechanisms of cellular changes in the BBB stimulated by PANS plasma.

Neetu Saini, PhD, Postoc
Advisor: Rosa Bacchetta
Department of Pediatrics - Stem Cell Transplantation

Generation of gut-specific-engineered Treg-like cells (GI-CD4LVFOXP3) for pediatric Crohn's disease treatment

Crohn's disease (CD) is a predominant form of inflammatory bowel disease characterized by chronic inflammation in the gut that affects millions of people worldwide. The CD is more prevalent in the pediatric population and less likely to respond to conventional therapies. Further, gut damage induced by uncontrolled inflammation often requires surgery and hospitalization and is a source of poor quality of life and suffering for many children as well as their families. Thus, pediatric CD patients urgently need treatment that could alleviate the disease symptoms with minimum side effects and support development and growth. We are developing gut-specific-engineered Treg-like cells (GI-CD4LVFOXP3), a patient-specific "living drug" that could control excess inflammation in the gut and support gut repair. Immunophenotyping of peripheral blood from CD patients showed the signature of inflammation and an increase in effector-T cells expressing gut-homing receptor a4b7+ integrins. We are generating GI-CD4LVFOXP3 Tregs from CD4+-T cells expressing gut-homing receptor a4b7+ integrins isolated from peripheral blood to promote gut

homing. GI-CD4LVFOXP3 showed expression of Tregs markers and gut repair molecule amphiregulin. Enteroids derived from CD patients but not from HD controls showed alteration in polarity, differentiation capacity, and transepithelial resistance. We aim to obtain data supporting the therapeutic efficacy of GI-CD4LVFOXP3 using autologous gut organoids, as preliminary evidence that GI-CD4LVFOXP3 will be a potent therapeutics for CD and co-culture of GI-CD4LVFOXP3 and enteroids will demonstrate the efficacy of these cells in reversing gut damage.

Friday, November 10, 2023
Merrill Hall
Session 3

3:00-3:20 PM	**Erice Meffre, PhD**, Professor of Immunology & Rheumatology, *TLR9 ligand sequestration by chemokine CXCL4 abrogates central B cell tolerance*
3:20-3:35 PM	**Qiwen Deng, PhD**, Postdoc, Wernig Lab, *Decoding the spatial transcriptomic landscape of diabetic nephropathy*
3:35-3:50 PM	**Rebeca Arroyo Hornero, PhD**, Postdoc, Idoyaga Lab, *A fraction of human plasmacytoid dendritic cells transdifferentiate into conventional dendritic cell type 2 following activation*

Erice Meffre, PhD, Professor
Department of Immunology & Rheumatology
TLR9 ligand sequestration by chemokine CXCL4 abrogates central B cell tolerance

Central B cell tolerance is believed to be regulated by B-cell receptor signaling induced by the recognition of self-antigens in immature B cells. Using humanized mice with defective MyD88, TLR7 or TLR9 expression, we demonstrate that TLR9/MYD88 are required for central B cell tolerance and the removal of developing autoreactive clones. We also show that CXCL4, a chemokine involved in systemic sclerosis (SSc), abrogates TLR9 function in B cells by sequestering TLR9 ligands away from the endosomal compartments where this receptor resides. The *in vivo* production of CXCL4 thereby impedes both TLR9 responses in B cells and the establishment of central B cell tolerance. We conclude that TLR9 plays an essential early tolerogenic function required for the establishment of central B cell tolerance and that correcting defective TLR9 function in B cells from SSc patients potentially by neutralizing CXCL4 may represent a novel therapeutic strategy to restore B cell tolerance.

Qiwen Deng, PhD, Postdoc
Advisor: Gerlinde Wernig
Department of Patholody
Decoding the spatial transcriptomic landscape of diabetic nephropathy

Diabetic nephropathy (DN) poses a significant challenge in the realm of chronic kidney diseases globally. However, the lack of specific treatments targeting renal fibrosis within DN stems from an incomplete comprehension of its progression. Addressing this gap, we embarked on a comprehensive study utilizing single-cell gene expression and spatial transcriptomic techniques. Analyzing patient biopsies through the 10x VISIUM-FFPE platform, we harnessed spatial transcriptomics and single-cell gene expression data to uncover cellular compositions and their spatial organization within kidney tissue.

Our investigation revealed intricate cellular niches. Within the glomerulus niche, we inferred a pseudotime trajectory from endothelial cells to pathogenic fibroblasts (IGKC+). We linked spatial cell composition information to cellular functions, detecting increased TGFß signaling activity in areas abundant in mesangial cells and fibroblasts. In the fibrotic niche, we observed strong dependencies between mesenchymal cells and leukocytes, highlighting the key role of macrophages in fibroblast activation. Subclustering fibroblasts and mesangial cells identified pathogenic fibroblasts (Fib 6) marked by IGKC and pathogenic mesangial cells (MES 2 & 3) marked by TMSB4X, MYL9, and ACTA2. Additionally, we discovered a novel innate immune checkpoint, CD63, highly expressed in DN samples and specifically overexpressed in Fib 6 and MES 2 & 3, supporting its crucial role in DN fibrogenesis. Our work thus provides a comprehensive molecular map of diabetic nephropathy, offering insights for advanced therapeutic strategies and improved disease management.

Rebeca Arroyo Hornero, PhD, Postdoc
Advisor: Juliana Idoyaga
Department of Microbiology & Immunology
A fraction of human plasmacytoid dendritic cells transdifferentiate into conventional dendritic cell type 2 following activation

Plasmacytoid dendritic cells (pDCs) are key players in antiviral immunity given their extraordinary ability to rapidly secrete large amounts of type I IFN (IFN-I) upon viral encounter. In addition, stimulated pDCs can activate T cells. Given this capacity to link innate and adaptive immune responses, pDCs were incorporated to the “dendritic cell family” twenty-four years ago. However, pDC identity as “dendritic cells” has been recently challenged because of the lack of evidence showing that these cells can indeed acquire functional features of conventional dendritic cells type 2 (cDC2s). Here, using high-dimensional approaches at the single-cell level, we demonstrate that upon activation, a fraction of pDCs have the capacity to secrete IFN-I, while others transdifferentiate into cells that are cDCs based on

their phenotype and transcriptome. Functionally, these pDC-derived cDC2s acquire the ability to uptake antigens and prime autologous naïve T cells, similar to bona fide cDC2s. These observations reveal an intrinsic plasticity within human pDCs, and their functional diversification following activation, i.e., innate cytokine secretion and induction of adaptive immune responses. Finally, our analysis unraveled that pDC transdifferentiation can be modulated by targeting the IFN-I and TNF signaling pathways. Altogether, our findings suggest that harnessing pDC functional plasticity, favoring IFN-I production or T cell activation, will offer a therapeutic approach in the context of disease pathogenesis.

Friday, November 10, 2023
Merrill Hall
Session 4

3:50-4:05 PM	**Grayson Rodriguez,** Immunology Graduate Student, Garcia Lab, *Rebalancing STAT signaling with non-natural cytokine receptor combinations to modulate immune cell functionality*
4:05-4:20 PM	**Vishnu Shankar**, Immunology Graduate Student, Davis and Mischel Labs, *Oxidative Stress is a shared characteristic of ME-CFS and Long COVID*
4:20-4:35 PM	**Alex Muselman**, Immunology Graduate Student, Engleman Lab, *Intracranial infection with gammaherpesvirus-68 primes microglia to a hyperinflammatory state and drives atypical EAE*

Grayson Rodriguez, Immunology Graduate Student
Advisor: Chris Garcia
Department of
Rebalancing STAT signaling with non-natural cytokine receptor combinations to modulate immune cell functionality

Recombinant cytokines have provided therapeutic benefits in a variety of disease contexts, but they only recapitulate the signaling properties of the natural parent cytokines. The plasticity of cytokine receptor JAK/STAT pairings enables the induction of new or modified cytokine receptor signals with non-natural STAT profiles, which could potentially exhibit enhanced therapeutic properties. Using a synthetic “orthogonal” IL-2Rb and common gamma chain receptor system (Sockolosky et al., Science 2018), we previously found that altered combinations of pSTAT5/pSTAT3/pSTAT1 exhibit emergent properties on engineered T cells over the pSTAT5-dominant signal of IL-2 (Kalbasi et al., Nature 2022). These engineered receptors enhanced stemness and mitigated T cell exhaustion in the setting of adoptive cell therapy. A limitation of this finding is that, while several natural cytokines exhibit strong pSTAT5 signaling on T cells (i.e. IL-2, IL-7), or pSTAT3 signaling (i.e. IL-21, IL-10), no natural cytokine exhibits simultaneous induction of pSTAT5, pSTAT3, and pSTAT1. Thus, we sought to endow IL-2-mediated pSTAT5-signaling on natural T cells with augmented pSTAT3 and pSTAT1 by engineering the IL-2 cytokine, eliminating the need to engineer cells. We created synthetic IL-2 analogs that rebalance pSTAT signaling profiles on natural cells through induced proximity of cytokine receptor combinations not normally formed in nature. We characterized the effects of combining the signals of common gamma chain family receptors in human T and NK cells. In CD4+ and CD8+ T cells, we observe differential modulation of pSTAT1, pSTAT3, and pSTAT5 signaling relative to the parent cytokines, as well as proliferation and cytokine production in response to stimulation. We also observe the mitigation of T cell exhaustion in long-term, repeated antigen challenge assays. In NK cells, we observe enhanced cytotoxicity and activation. These findings provide the foundation for a new catalog of engineered cytokine therapeutics based on compelling new receptor combinations.

Vishnu Shankar, Immunology Graduate Student
Advisors: Mark Davis, Department of Microbiology & Immunology
and Paul Mischel, Department of Pathology

Oxidative Stress is a shared characteristic of ME-CFS and Long COVID

Authors: Vishnu Shankar[1], Ellis J. Curtis[9], Basil Michael[3], Julie Wilhelmy[2], Ronald Davis[2], Michael Snyder[3], William Robinson[6,7], Paul Mischel[9,10], Sadasivan Shankar[4,5], Hector Bonilla[8], Mark M. Davis[11, 12, 13†]

[1]Program in Immunology, Stanford University School of Medicine, Stanford, California 94305 USA.
[2]ME/CFS Collaborative Research Center at Stanford, Stanford Genome Technology Center, Stanford University School of Medicine, Palo Alto, California, United States of America
[3]Department of Genetics, Stanford University School of Medicine, Stanford, California 94305 USA.
[4]SLAC National Accelerator Laboratory, Menlo Park, CA
[5]Materials Science and Engineering, Stanford University, CA, USA
[6]Division of Immunology and Rheumatology, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA
[7]The Geriatric Research, Education, and Clinical Center (GRECC), VA Palo Alto Health Care System, Palo Alto, CA, USA
[8]Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA
[9]Department of Pathology, Stanford University School of Medicine, Stanford University, Stanford, CA, USA
[10]Sarafan ChEM-H, Stanford, CA, USA
[11]Institute of Immunity, Transplantation and Infection, Stanford University, Stanford, CA 94305, USA
[12]Department of Microbiology and Immunology, Stanford University, Stanford, CA 94305, USA
[13]The Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305, USA

More than 65 million individuals worldwide are estimated to have Long COVID (LC), a complex multisystemic condition, where patients of all ages report fatigue, post-exertional malaise, and other symptoms resembling myalgic encephalomyelitis or chronic fatigue syndrome (ME-CFS). With no current treatments or reliable diagnostic markers, there is an urgent need to define the molecular underpinnings of these conditions. By comparing bioenergetic parameters among healthy controls (n=16), ME-CFS (n=15), and LC donors (n=15) peripheral blood mononuclear cells, we find both ME-CFS and LC donor lymphocytes exhibit signs of elevated oxidative stress, especially among the memory compartment. Using a combination of flow cytometry, bulk RNA-seq analysis, mass spectrometry, and systems chemistry analysis, we also find aberrations in ROS clearance pathways, including glutathione, mitochondrial superoxide clearance, hydrogen peroxide detoxification, and lipid oxidative damage. Further experiments reveal that oxidative stress is positively correlated with T cell proliferation upon stimulation, where CFS and LC donor T cells hyperproliferate compared to healthy controls, suggesting a possible source of patient fatigue. Together, our results highlight a potential molecular diagnostic for ME-CFS and LC, pinpoint specific pathways that are dysregulated in chronically fatigued donors, and identify a targetable source of patient fatigue.

Alex Muselman, Immunology Graduate Student
Advisor: Ed Engleman
Department of Pathology, and of Immunology & Rheumatology

Intracranial infection with gammaherpesvirus-68 primes microglia to a hyperinflammatory state and drives atypical EAE

Multiple sclerosis (MS) is the most common demyelinating disorder of the central nervous system (CNS), driven by inflammation targeting the myelin sheaths of neurons. Epidemiological evidence suggests that Epstein-Barr Virus (EBV) may trigger MS. Molecular mimicry has been implicated as one potential mechanism in which EBV infection leads to MS; however, whether and how such molecular mimicry results in inflammation is unknown and the possibility exists that immune cells in addition to infected B cells contribute to disease development. Microglia have been found to be infected by EBV in MS brains, but the role of EBV-infected microglia in MS is unknown. To address this question in a mouse model, we used the murine gammaherpesvirus-68 (MHV-68) model, which establishes latency and recapitulates the pathology of human EBV infection. While intraperitoneal MHV-68 predominantly infects and establishes latency in B cells, intracranial MHV-68 predominantly infects and establishes latency in microglia. Once latency is established, microglia in intracranially infected mice exhibit elevated MHC-II and Fc gamma receptor expression and secrete large amounts of pro-inflammatory cytokines when stimulated, indicative of microglial priming. To investigate the potential role of primed microglia in autoimmunity, we induced EAE one month after MHV-68 intracranial infection. The latently infected mice displayed impaired coordination and balance, consistent with atypical EAE. Our findings demonstrate that latent infection in the brain with a virus that primes microglia towards a hyperinflammatory state contributes to the development of an MS-like disease. Involvement of human microglia with EBV may similar contribute to the development of MS.

Saturday, November 11, 2023
Merrill Hall
Session 5

9:00-9:20 AM	**David Lewis, MD,** Professor of Pediatrics - Immunology and Allergy, *Using CRISPR-edited induced pluripotent stem cells (iPSCs) to define mechanisms of disease in Schimke Immuno-Osseous Dysplasia (SIOD), a monogenic primary immunodeficiency and complex disease*
9:20-9:35 AM	**John Hickey, PhD**, Postdoc, Nolan Lab, *Associations of immune hubs in metaplastic progression to adenocarcinoma revealed by high-speed multiomic spatial phenotyping of FFPE human samples*
9:35-9:50 AM	**Kalani Ratnasiri**, Immunology Graduate Student, Blish and Khatri Labs, *Conserved monocyte responses to acute RNA viruses*

David Lewis, MD, Professor of Pediatrics - Immunology & Allergy

Using CRISPR-edited induced pluripotent stem cells (iPSCs) to define mechanisms of disease in Schimke Immuno-Osseous Dysplasia (SIOD), a monogenic primary immunodeficiency and complex disease

Viswanath Gunda, Jordan T. Spatz, Vasavi Ramachandran, David B. Lewis. Department of Pediatrics, Stanford Immunology, Institute for Immunity, Transplantation, and Infection, Stanford University School of Medicine

Schimke Immuno-Osseous Dysplasia (SIOD) is a T-cell immunodeficiency that invariably also includes a unique phenotype of non-immune-mediated organ involvement, including progressive kidney failure, impaired skeletal growth resulting in dwarfism, and abnormalities of the endothelium (blood vessel lining cells) associated with atherosclerotic neurovascular disease. SIOD is due to biallelic mutations in the *SMARCAL1*(SWI/SNF Related, *M*atrix Associated, *A*ctin Dependent *R*egulator Of *C*hromatin, Subfamily *A* Like-1) gene, which encodes a large nuclear protein known to facilitate genomic DNA replication by helping restart stalled DNA replication forks and to be important in telomere maintenance. A recent major breakthrough in the treatment of the immunodeficiency and renal disease of SIOD uses the sequential transplant of hematopoietic stem cells and a kidney from the same donor mother or father. Consistent with SIOD being an autosomal recessive disorder, these parental donors are obligate heterozygous carriers for a damaging *SMARCAL1* mutation but are asymptomatic and do not manifest the clinical manifestations of the biallelic disease. Unfortunately, the double transplant procedure does not appear to reduce the risk of neurovascular complications or to normalize skeletal growth. How biallelic SMARCAL1 deficiency results in this unique and complicated disease phenotype remains a major medical mystery, and solving it might lead to treatments for the complications of SIOD that allogeneic transplantation cannot correct. We hypothesize that defective DNA replication and telomere maintenance result in a common downstream perturbation, such as intermittent metabolic stress, that selectively impairs the function of the cell types/tissues involved in SIOD. As murine gene knockout models have failed to recapitulate any of the major features of human SIOD, we are testing this hypothesis using differentiated cell types and organoids derived from human induced pluripotent stem cells (iPSCs).

Taking advantage of recent progress in highly efficient CRISPR editing of stem cells using homology-directed repair (HDR)templates, we have selectively targeted the *SMARCAL1* gene in iPSCs to create two different disease models. In the first, the SMARCAL1 gene has been biallelically edited to encode a carboxy-terminal protein tag. This tag confers sensitivity to ubiquitination and protein destruction after the incubation of cells with a nontoxic small molecule drug. This allows the differentiation of the cell type or organoid of interest in the presence of normal SMARCAL1 protein levels and an assessment of the impact of acute or chronic depletion of SMARCAL1 protein in a cell type/tissue-specific manner. In the second model, iPSCs from SIOD patients, carrier parents, or normal controls are undergoing HDR targeting to create isogenic iPSC lines that differ only in the functional status of their *SMARCAL1* gene (i.e., wildtype, heterozygous or homozygous for damaging mutations). Both models should be helpful in determining why SMARCAL1 deficiency results in T-cell immunodeficiency (human thymic organ culture system), endothelial and neurovascular complications (endothelial cell differentiation and vascular tube formation), and other disease manifestations. These models should also be valuable in identifying potential small molecule approaches for disease amelioration. Our very first studies of endothelial differentiation strongly from iPSCs strongly suggest that normal SMARCAL1 protein levels are important for the overall efficiency of this process, with a previously unappreciated adverse impact of SMARCAL1 haploinsufficiency/SIOD carrier status on this differentiation.

John Hickey, PhD, Postdoc
Advisor: Garry Nolan
Department of Clinical Departments

Associations of immune hubs in metaplastic progression to adenocarcinoma revealed by high-speed multiomic spatial phenotyping of FFPE human samples

Chronic inflammation resulting from recurrent tissue injury can disrupt tissue homeostasis and contribute to tumorigenesis in organs like the esophagus, lung, stomach, and intestine. However, only some patients with inflammation-induced epithelial metaplasia progress to cancer. Understanding the link between inflammation (immune cells) and stroma (mesenchymal cells) creating an environment conducive to epithelial metaplasia is crucial for prevention and treatment.

We utilized Phenocycler technology to characterize 405 tissue samples from 26 Barrett's esophagus (BE) patients with metaplasia who haven't progressed to adenocarcinoma and 6 BE patients who have progressed, alongside 23 samples from various disease states. Phenocycler Fusion enabled rapid spatial phenotyping of whole slide FFPE samples using a 56-antibody panel and 100+ RNA molecules in 1.1 million cells, identifying 57 cell types including immune cells and metaplastic epithelium.

CODEX multiplexed imaging revealed epithelial misdifferentiation into an intestinal-like state and subsequent dedifferentiation within cancer. Multicellular neighborhoods associated with pathological tissue were identified, showcasing changes in cellular microenvironment to an intestinal-like stromal environment and increased diversity with disease progression.

Progressors exhibited increased CD4+ Treg cells, neutrophils, and decreased plasma cell multicellular neighborhoods compared to non-progressors. Single-cell transcriptomics revealed transitions in fibroblast populations with disease progression, influencing immune cell recruitment and maintenance of inflamed-epithelial cellular neighborhoods.

This approach could guide novel therapies targeting stroma to reverse cellular rearrangements governing

dedifferentiation or provide risk stratification. Future work involves integrating this data with scRNAseq and ECM proteomics to further understand cross-talk between fibroblasts, immune, and epithelial cells.

Kalani Ratnasiri, Immunology Graduate Student
Advisors: Catherine Blish, Department of Infectious Diseases and Purvesh Khatri, Department of (Research), Medicine, and of (Research), Biomedical Data Science

Conserved monocyte responses to acute RNA viruses

The 21st century has seen five virus-driven pandemics. Despite emerging viruses constantly threatening global health, we remain largely unprepared for the next pandemic. Previous analyses have found that emergency myelopoiesis and monocyte dysregulation correlate with severe disease in COVID-19. Here, we leverage proteomic and transcriptional single-cell profiling studies across acute RNA viral diseases to identify conserved and diverse features of disease. We identify shifts in monocyte populations towards increased CD14+ monocytes across viral infection of humans and macaques by different viral species – including Lassa, Ebola, Marburg, influenza, Zika, and dengue. We integrate scRNA-seq data across all publically available datasets that profile non-COVID-19 viral diseases that include infection with dengue, influenza or RSV. Using this integrated data, we demonstrate that there is a conserved shift in monocyte phenotype that correlates with severity, defined by markers of myeloid-derived suppressor-like cells and bacterial sepsis-induced monocytes. Utilizing over 2000 publicly available samples, we identify that this signature is conserved upon disease severity in Ebola-infected rhesus macaques and robustly detected in bulk RNA-seq studies of blood cells from both human and macaques infected with diverse viruses. We expect that utilizing single-cell resolution through integrative systems immunology approaches will identify dysfunctional markers shared across different viral infections and, in the process, yield detailed insights to the underlying monocyte-specific pathological processes that may be targeted in a broad-spectrum manner.

Saturday, November 11, 2023
Merrill Hall
Session 6

10:05-10:20 AM	**Dongeon Kim, PhD**, Postdoc, Nicholls Lab, *Abnormal lymphatic S1P signaling aggravates lymphatic dysfunction and tissue inflammation*
10:20-10:35 AM	**Brenda Velasco**, Immunology Graduate Student, Shizuru Lab, *The effects of anti-CD117 monoclonal antibody on the bone marrow microenvironment*
10:35-10:50 AM	**Simon Borna, PhD**, Postdoc, Bacchetta Lab, *Analyses of Treg plasticity and TCR repertoire autoreactivity in patients with FOXP3 mutation*

Dongeon Kim, PhD, Postdoc
Advisor: Mark Nicholls
Department of Pulmonary, Allergy & Critical Care Medicine
Abnormal lymphatic S1P signaling aggravates lymphatic dysfunction and tissue inflammation

BACKGROUND: Lymphedema is a global health problem with no effective drug treatment. Enhanced T cell immunity and abnormal lymphatic endothelial cell (LEC) signaling are promising therapeutic targets for this condition. Sphingosine-1-phosphate (S1P) mediates a key signaling pathway required for normal LEC function, and altered S1P signaling in LECs could lead to lymphatic disease and pathogenic T cell activation. Characterizing this biology is relevant for developing much-needed therapies.

METHODS: Lymphedema was induced in mice by surgically ligating the tail lymphatics. Lymphedematous dermal tissue was assessed for S1P signaling. To verify the role of altered S1P signaling effects in lymphatic cells, LEC-specific S1pr1-deficient (S1pr1LECKO) mice were generated. Disease progression was quantified by tail-volumetric and -histopathological measurements over time. Mice and humans LECs, with S1P signaling inhibition, were then co-cultured with CD4 T cells, followed by an analysis of CD4 T cell activation and pathway signaling. Animals were treated with a monoclonal antibody specific to P-selectin to assess its efficacy in reducing lymphedema and T cell activation.

CONCLUSION: This study suggests that reduction of the LEC S1P signaling aggravates lymphedema by enhancing LEC adhesion and amplifying pathogenic CD4 T cell responses. P-selectin inhibitors are suggested as a possible treatment for this pervasive condition.

Keywords: Lymphedema, Lymphatic endothelial cells (LECs), Sphingosine-1-phosphate receptor 1 (S1PR1), P-selectin, and CD4 T cells.

Brenda Velasco, Immunology Graduate Student
Advisor: Judy Shizuru
Department of Blood & Marrow Transplantation, and of Pediatrics - Stem Cell Transplantation

The effects of anti-CD117 monoclonal antibody on the bone marrow microenvironment

CD117 is a molecule expressed on hematopoietic stem and progenitor cells (HSPC) and is the receptor for stem cell factor (SCF). The interaction between SCF and CD117 is required for HSPC survival and proliferation. The monoclonal antibody (mAb) against mouse CD117, also known as ACK2, depletes HSPC, thereby permitting donor cells to engraft. Studies have shown that anti-CD117 treatment permits HSPC engraftment in immune-deficient mice, but not immunocompetent congenic mice. We have observed that anti-CD117 conditioning permits donor cell engraftment in T, B and NK (RAG2-/-cγ-/-) deficient mice as well as T cell deficient TCRβ-/- but not in B cell deficient muMt- mice, suggesting T cells act beyond classical immune rejection in resisting HSPC engraftment following anti-CD117 conditioning (Fig. 1).

Our observations lead us to hypothesize that resident T cells in the host bone marrow can inhibit donor HSCs from engrafting by affecting the marrow microenvironment. Specifically, that higher levels of proinflammatory cytokines, pre- and post-conditioning with anti-CD117 antibody, in WT and T cell-sufficient mice, play a significant role in the ability of HSCs to engraft. We show that proinflammatory cytokines are found at higher levels in WT mice, T cell-sufficient mice, and in mouse models of Type 1 diabetes and Sickle Cell Disease, compared to T cell deficient mice, at baseline and following anti-CD117 conditioning.

To directly study the effect of proinflammatory cytokines on engraftment in immunocompetent we performed HSCT from WT mice into T cell deficient TCRβ-/- mice treated with anti-CD117 and recombinant proinflammatory cytokines. We found that exogenous proinflammatory cytokines are sufficient to significantly inhibit donor cell engraftment in T cell deficient mice, supporting this idea that proinflammatory cytokines play an important role in donor cell rejection.

In the setting of altered levels of proinflammatory cytokines we observed modulation of CD47 expression on bone marrow cells. CD47 is a molecule that functions on the SIRPa axis and prevents phagocytosis. Alterations of CD47 expression following anti-CD117 conditioning mice can impact clearance of donor HSC.

Conditioning of mice with anti-CD117 antibody also leads to higher levels of B cell and granulocyte apoptosis and necrosis in TCRβ-/- mice, compared to WT controls, which may also be key factor that allows anti-CD117 conditioning to function as a sole conditioning agent in T cell-deficient mice. Our data suggests that resident T cells in the host bone marrow can inhibit donor HSCs from engrafting through non-classical immune rejection, by secreting cytokines, chemokine and soluble factors that lead to an altered the marrow microenvironment.

Simon Borna, PhD, Postdoc
Advisor: Rosa Bacchetta
Department of Pediatrics - Stem Cell Transplantation

Analyses of Treg plasticity and TCR repertoire autoreactivity in patients with FOXP3 mutation

Immune dysregulation polyendocrinopathy enteropathy X linked syndrome (IPEX) is a prototypical autoimmune disease caused by loss of Treg function due to a mutation in the FOXP3 gene. Although much work has been done in the murine models of FOXP3 deficiency, there is very little evidence about Treg plasticity and no evidence about TCR autoreactivity in FOXP3-deficient humans. Here, we combined cytometric and epigenetic data, single-cell RNA/protein/TCR profiling, and bulk TCR sequencing to shed light on Treg plasticity and TCR autoreactivity in IPEX. We found that IPEX Treg are expanded, and they separate into two memory populations. The first population is the typical Treg population; it is present in normal frequencies and is composed of CD25highCD127low cells. The second population of Treg is specific for IPEX; these Treg are clonally related to the typical Treg population but they have reduced or lost expression of the typical Treg markers including CD25 and FOXP3. We named the atypical Treg population loss-of-identity Treg and we further showed that they have autoreactive TCR repertoire and signature of TNFa signaling indicating that they were exposed to the autoimmune inflammatory environment. Moreover, our functional tests revealed that IPEX Treg gain Th2 Teff-like phenotype thus indicating that IPEX Treg are a source of autoreactive T cells in IPEX. In addition, we observed increased TCR autoreactivity in Teff compartment. Collectively, these data suggest the sources of autoreactive T cells in IPEX consist of both expanded autoreactive Teff, and unstable Treg.

Sunday, November 12, 2023
Merrill Hall
Session 7

9:00-9:20 AM	**Derick Okwan, MD, PhD**, Assistant Professor of Pathology, *Role of neutrophils in brown adipose thermogenesis*
9:20-9:35 AM	**Adonis Rubio**, Immunology Graduate Student, Barnes Lab, *Engineering bispecific antibodies that recognize the SARS-CoV-2 Spike glycoprotein N-terminal and receptor binding domains*
9:35-9:50 AM	**Ana Jimena Pavlovitch-Bedzyk**, Immunology Graduate Student, Davis Lab, *Immune competent air liquid interface skin organoids reveal monkeypox dynamics*

Derick Okwan, MD, PhD, Assistant Professor
Department of Pathology
Role of neutrophils in brown adipose thermogenesis

Neutrophils have long been considered short-lived innate immune cells that solely combat infections. Yet, accumulating evidence indicates that in the absence of infection or inflammation, neutrophils patrol nearly all tissues, where there may modulate local homeostatic processes. Here we show that neutrophils infiltrate brown adipose tissue (BAT), exit the intravascular space, associate with adipocytes, and undergo transcriptional reprogramming for a specialized role in adaptive thermogenesis. Pharmacologic, antibody-mediated, or genetic depletion of neutrophils compromise adaptive thermogenesis by cold challenge. BAT neutrophils utilize an ancient anti-microbial autophagic pathway, via ATG14, to facilitate the transfer of adipocyte mitochondria. These findings raise the intriguing possibility of arming neutrophils to improve metabolic homeostasis, a critical underpinning of most modern chronic diseases.

Adonis Rubio, Immunology Graduate Student
Advisor: Chirstopher Barnes
Department of Biology
Engineering bispecific antibodies that recognize the SARS-CoV-2 Spike glycoprotein N-terminal and receptor binding domains

Neutralizing antibodies have been comprehensively investigated and shown to be effective for the prevention and treatment of COVID-19. However, the emergence of variants of concern (VOCs), such as the Omicron sub-lineages, has rendered most antibody therapeutics ineffective. Bispecific antibodies (bsAbs) hold great promise as a solution to antigenic drift, offering various advantages over current conventional monoclonal antibodies or cocktail therapeutics, such as increased resistance to viral evasion and enhanced avidity of viral antigen binding. Here, we generated bsAbs targeting two regions of the SARS-CoV-2 Spike glycoprotein: the receptor binding domain (RBD) and the N-terminal domain (NTD). To generate our bsAb, we determined a 3.0 Å cryo-EM structure of a cross-reactive neutralizing NTD-specific antibody and identified RBD-specific antibodies whose binding geometries were compatible with an IgG-like bsAb format. Using these compatible NTD-RBD Ab pairs, we engineered a panel of bsAbs with dual specificity for the NTD and RBD that utilized three distinct bsAb formats. Preliminary data demonstrated successful expression of our bsAbs and confirmed their capacity to bind to their respective epitopes in comparison to the original antibodies. Of note, we identified two candidate bsAbs that exhibit increased in vitro neutralizing activity against one of the most divergent VOCs, XBB.1.5, relative to the parental monoclonal Abs or a cocktail of the two, highlighting the potential utility of our bsAb constructs as novel antibody therapeutic candidates against emerging VOCs.

Ana Jimena Pavlovitch-Bedzyk, Immunology Graduate Student
Advisor: Mark Davis
Department of Microbiology & Immunology

Immune competent air liquid interface skin organoids reveal monkeypox dynamics

We have developed a model system that recapitulates nearly all elements of native human skin biology. Currently, the available systems to study skin biology are limited to animal models or other incomplete in vitro culture systems. Animal models suffer from well known differences between human and model organism biology, while the in vitro culture systems fail to incorporate critical elements such as stromal and immune cells. Our newly developed system contains all the native elements of human skin, allowing for critical studies of human skin biology. As assessed by histology, immunohistochemistry, and single cell sequencing the culture system reproduces in near native proportions the cells that make up the epidermis and dermis, including resident immune cells, fibroblasts, keratinocytes, as well as the many other diverse cell types present in human skin. As a major barrier to infection, human skin must repel and respond to a diverse set of stimuli. In a first in class proof of principle we demonstrate active monkeypox infection within the culture system with correspondent viral cytopathic effects. Current work is aimed at utilizing this powerful tool to increase our understanding of monkeypox virus dynamics within the skin and the associated immune response that leads to dissemination and ultimately virus clearance.

Sunday, November 12, 2023
Merrill Hall
Session 8

11:15-11:30 AM	**Surbhi Sharma, PhD**, Postdoc, Mellins Lab, *Anakinra, an IL-1 inhibitor, alters peptide interactions with HLA-DR15, a risk haplotype of sJIA/DRESS*
11:30-11:45 AM	**Hayley Raquer**, Immunology Graduate Student, Idoyaga Lab, *Ontogeny impacts Langerhans cell functional properties*
11:45 AM-12:00 PM	**Cameron Bader, PhD**, Postdoc, Meyer Lab, *Single CD4 T cell phenotypic structure and oligoclonal polarization favor FOXP3 and HELIOS over IFNG and proliferative genes in Orca T patients without GVHD*
12:00-12:15 PM	**Noor Hussein, PhD**, Postdoc, Mellins Lab, *Regulatory T cells subsets in Pediatrics Acute Onset Neuropsychiatric Syndrome (PANS)*

Surbhi Sharma, PhD, Postdoc
Advisor: Elizabeth Mellins
Department of Pediatrics - Human Gene Therapy
Anakinra, an IL-1 inhibitor, alters peptide interactions with HLA-DR15, a risk haplotype of sJIA/DRESS

Severe delayed drug hypersensitivity reactions (DHR) with eosinophilia and systemic symptoms (DRESS), sometimes including severe parenchymal lung disease, are observed in some patients with systemic juvenile idiopathic arthritis (sJIA) treated with inhibitors of Interleukin-1 (IL-1: anakinra) or interleukin-6 (IL-6: tocilizumab). Analysis of HLA genotypes in sJIA/DRESS showed an enrichment of HLA-DRB1*15:01 (and its haplotype). One current model of HLA-linked DHRs postulates drugs interact either with HLA-peptide complexes or their components leading to immune activation. We hypothesized that IL-1 and IL-6 inhibitors lead to DRESS by altering HLA-DRB1*15:01 restricted antigen presentation in sJIA patients. By performing in-vitro peptide competition assays, we found a statistically significant, dose-dependent decrease in indicator peptide binding to HLA-DRB1*15:01 in the presence of anakinra. By testing additional alleles within the haplotype (DQ0602, DRB5*01:01), structurally similar allotypes (DRB1*15:02, DRB1*15:03) and unrelated allele (HLA-DRB1*04:01), we concluded that anakinra affects peptide exchange on HLA class II molecules to varying degrees. We further identified the culprit molecule in the drug to be the IL-1 receptor antagonist, the active ingredient, and not its excipients, including polysorbate-80. We also observed that anakinra affects peptide exchange more prominently at acidic pH than at the neutral pH, and no appreciable binding of anakinra with DRB15:01 was detected at neutral pH. Our findings suggest a model in which anakinra (IL-1Ra) interacts with risk-associated HLA molecules in the MHC-II presentation pathway in a cell, with a potential to alter the global HLA-DRB1*15:01-restricted peptidome presented to CD4+ T cells, leading to DRESS episodes in sJIA patients.

Hayley Raquer, Immunology Graduate Student
Advisor: Juliana Idoyaga
Department of Microbiology & Immunology

Ontogeny impacts Langerhans cell functional properties

Langerhans cells (LCs) are unique myeloid cells of the epidermis with features of both macrophages and dendritic cells. Similar to other tissue-associated macrophages, LCs are embryonically derived. However, different from other embryonically derived macrophages, LCs are able to migrate to skin-draining lymph nodes and present antigen for priming of antigen-specific T cells, a functional property usually attributed to dendritic cells (DCs). This constant homeostatic migration to LN requires LC maintenance within the epidermal niche, which is thought to be dependent on self-renewal or monocyte differentiation. Here, we used models of complete or partial LC depletion to investigate the mechanisms that these cells are using for their maintenance in the epidermis during homeostasis. We found that a complete ablation of LCs at steady-state results in the presence of epidermal monocytic LCs (moLCs) that are highly proliferative. Despite their proliferation potential, moLCs are unable to reconstitute the epidermis, even after six months post-embryonic LC (eLC) depletion. Instead, moLCs quickly migrate and refill the skin-draining lymph node niche. The superior capacity of moLCs to migrate to lymph nodes is associated with their higher expression of activation and migration markers such as MHCII, CCR7, CD86, and PDL1. Moreover, moLCs are also more efficient than eLCs at capturing skin-associated pathogenic antigens, which may explain their superior migratory capabilities. Altogether, our data suggest that ontogeny of LCs plays a role in their function during homeostasis.

Cameron Bader, PhD, Postdoc
Advisor: Everett Meyer
Department of Medicine - Blood & Marrow Transplantation and of Pediatrics - Stem Cell Transplantation

Single CD4 T cell phenotypic structure and oligoclonal polarization favor FOXP3 and HELIOS over IFNG and proliferative

Background: Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative therapy for hematopoietic malignancies. We have established a novel HSCT regimen – termed Orca T – which appears to reduce the incidence of graft-versus-host disease (GVHD). Here, we compared single-cell RNA sequencing (scRNA-seq) of sorted lymphocyte populations collected from Orca T or SOC patients to identify an immune signature that could identify patients who would subsequently develop aGVHD or cGVHD.

Methods: Blood was collected from patients 16-21 days after HLA-matched HSCT. Tcons or Tregs were sorted for targeted immune-related gene and VDJ single-cell sequencing using the Rhapsody platform.

Results: We sequenced a total of 212,166 cells across 8 patients. After clustering of Tcons and Tregs, we observed that

SOC had a higher frequency of Tcons marked by CCR7 and TXK expression and Tregs expressing either IFNG, IL32, and proliferative genes or LAG3. Meanwhile, Orca T patients were found to have a higher frequency of IKZF2 – encoding Helios – and CCR8 expressing Tregs. Among both Tcons and Tregs, enriched T cell receptor clonotypes from SOC were found in clusters marked by expression of IFNG and proliferative genes while Orca T enriched clonotypes where GVHD did not develop associated more frequently with clusters expressing FOXP3 and IKZF2. We hypothesize that expanded clonotypes found in proinflammatory/proliferating clusters may be a marker of ongoing alloreactivity.

Conclusions: Our findings indicate that combined scRNA-seq and VDJ sequencing can allow the identification of potentially alloreactive clonotypes early post-HSCT by mapping the expansion of clonotypes onto UMAP single cell clustering.

Noor Hussein, PhD, Postdoc
Advisor: Elizabeth Mellins
Department of Pediatrics - Human Gene Therapy

Regulatory T cells subsets in Pediatrics Acute Onset Neuropsychiatric Syndrome

Pediatric Acute Onset Neuropsychiatric Syndrome (PANS) is a relapsing and remitting disorder that constitutes 10-20% of pediatric OCD. To date, there is no specific therapy for PANS, though some patients respond to anti-inflammatory and/or anti-psychotic drugs. PANS is associated with neuroinflammation and autoimmunity. Regulatory T cells (Tregs) are key inhibitors of autoimmunity and inflammation. They are hypothesized be involved in OCD immunopathogenesis However, the role of Tregs in PANS remains largely unexplored. Previously, we evaluated the percentages of Tregs in peripheral blood of 10 children with paired samples from PANS new-onset flare and improved status, and 6 age-sex-matched healthy controls (HCs). Our results showed a trend towards increased frequency of CD4+CD127low/-CD25high in PANS flare patients compared to HCs as well as a decrease in paired samples after PANS improvement. Using additional samples from PANS patients with different trajectory (i.e., flare on a good baseline) n=18, we found similar trend. Additionally, we found a significant increase in DNA demethylation at Treg-specific epigenetic control region (TSDR), indicating an increase in bona fide Tregs. We also found that % of CD39+, GITR+, GARP+ (markers of highly active and suppressive Tregs) within Tregs were significantly higher in PANS flare compared to HCs and clinically improved PANS ($p = 0.01$, 0.02, and 0.05, respectively). We hypothesize that significant increase in circulating Tregs subsets in PANS flare signals the development of an immunosuppressive response to the neuroinflammation of PANS flares. Further research is ongoing to determine the functional and transcriptional profile of Tregs during PANS flare and improvement.

AGENDA | POSTER SESSION

Stanford MEDICINE Immunology
School of Medicine

Saturday, November 11, 2023 at 8:30 – 11:00 PM
Merrill Hall
Poster session

Presenter & Title
Koji Abe, PhD, Basic Life Research Scientist, Maecker Lab, *A multi-platform assessment of high-sensitivity protein assays for inflammatory markers from a COVID-19 cohort*
Rizwan Ahmad, PhD, Senior Research Scientist, Utz Lab, *Coexisting networks of TCRs and BCRs with mosaic public CDR3 sequences comprising conserved junctional motifs and their implications in immune responses and autoimmune disorders*
Gabe Barron, Immunology Graduate Student, Howitt Lab, *The role of follicular-associated tuft cells on Peyer's patch dynamics*
Leslie Chan, Immunology Graduate Student, Blish Lab, *Functional and transcriptional investigation of NK responses in COVID-19 breakthrough infections*
Izumi de los Rios Kobara, Immunology Graduate Student, Blish Lab, *High natural killer cell proportion and immune activation correlate with low neutralization breadth in wild type SARS-CoV-2 infection*
Markus Diehl, Immunology Graduate Student, Engleman and Reticker-Flynn Labs, *Neutrophil-activating therapy initiates systemic response to metastasis*
Xiaowen Ding, PhD, Postdoc, Mellins Lab, *Response of HLA-DRB1*15-restricted T cells in anakinra-associated delayed hypersensitivity reactions*
Maïgane Diop, Immunology Graduate Student, Gaudillière and Blish Labs, *Uterine natural killer cells modulate endometrial growth and persistence in endometriosis*
Tyson Holmes, PhD, Statistical Director, Human Immune Monitoring Center, *Multiple regression methods for immunologists*
Shani Jahanbani, Research Associate, Robinson Lab, *Increased macrophage phagocytic activity with TLR9 agonist conjugation of an anti- Borrelia burgdorferi monoclonal antibody*
Madeline Lee, Immunology Graduate Student, Blish Lab, *NK cell-monocyte crosstalk underlies NK cell activation in severe COVID-19*
Claudia Macaubas, Research Scientist, Mellins Lab, *Natural killer (NK) cells in patients with Pediatric Acute-onset Neuropsychiatric Syndrome (PANS)*
Evan Maestri, Immunology Graduate Student, Khatri Lab, *Transcriptomic analysis of lupus reveals two disease endotypes*
Jae Seung Moon, PhD, Postdoc, Robinson Lab, *Clonally expanded cytotoxic CD8+ T cells target citrullinated antigens in ACPA+ rheumatoid arthritis*
Jason Nideffer, Immunology Graduate Student, Jagannathan and Roncarolo Labs, *The pediatric CD4+ T cell response to malaria*
Trung Pham, MD, PhD, Assistant Professor of Pediatrics – Infectious Diseases, *Uncovering mechanisms of tissue immunity and immunophysiology during persistent infection*
Ruoxi Pi, PhD, Postdoc, Blish Lab, *Engineering natural killer (NK) Cells to target Human Immunodeficiency Virus (HIV) reservoirs*

Andrea Reitsma, Lab Manager/Clinical Research Coordinator and **Ayantika Sen**, Postdoc, Krams and Martinez Labs, *Longitudinal immune profiling of whole blood reveals sustained expansion of γδ T cells in children with multisystem inflammatory syndrome*

Sarah Sackey, Immunology Graduate Student, Blish Lab, *Establishing tonsil and splenic organoids as a model of HIV infection in secondary lymphoid tissues*

Aiswarya Sethumadhavan, PhD, Postdoc, Meffre Lab, *Assessment of the tolerogenic role of B cells during pregnancy*

Vishnu Shankar, Immunology Graduate Student, Davis and Mischel Labs, *Using single-cell RNA sequencing data on human patient biopsies to define new therapeutic targets in follicular lymphoma*

Sasa Vasilijic, PhD, Senior Research Scientist Basic Life Scientist, Stankovic Lab, *Identification of immune-related candidate biomarkers in plasma of patients with sporadic vestibular schwannoma*

Clarence Rachel Villanueva, MS, Life Science Research Professional I, Meffre Lab, *Anti-B cell therapy may prevent the production of autoreactive B cells in relapsing remitting multiple sclerosis*

Edward Vizcarra, PhD, Postdoc, Martinez Lab, *Characterization of EBV-specific peripheral T cells from pediatric transplant patients with elevated EBV DNAemia or controlled infection*

Oliver Wirz, PhD, Postdoc, Boyd Lab, *Systematic multi-antigen analysis of SARS-CoV-2-specific B cells defines distinct memory and functional subsets in blood and lymphoid tissues of patients and vaccinees*

Koji Abe, PhD, Basic Life Research Scientist
Advisor: Holden Maecker
Department of Microbiology and Immunology

A multi-platform assessment of high-sensitivity protein assays for inflammatory markers from a COVID-19 cohort

In this study, we conducted a comprehensive evaluation of three distinct immunoassay platforms designed for the simultaneous detection of multiple cytokines and associated proteins. These platforms utilize antibody pairs to capture and detect target proteins. Our assessment focused on three performance metrics - detectability, correlation, and differential expression - using serum samples from the NIH IMPACC study. The platforms under scrutiny were the fluorescent bead-based Luminex assay, the proximity extension-based Olink assay, and the novel proximity ligation assay platform, Alamar NULISAseq. Our findings yield useful insights. The Alamar platform exhibited the highest overall detectability, followed by Olink and Luminex. Moreover, protein measurement correlations between Alamar and Olink were generally stronger than those observed between either of these platforms and Luminex. Notably, detectability discrepancies across platforms often translated into differential expression disparities. It's worth noting that while high detectability enhances the potential to identify meaningful biological differences, it doesn't guarantee it. Through our study, we offer valuable insights into the comparative performance of these assays. This enhances our understanding of their strengths and limitations, particularly when applied to complex biological samples, as exemplified by the sera from the COVID-19 cohort. Our research contributes to the refinement of protein assay selection and data interpretation for future studies involving intricate disease cohorts. (Manuscript in preparation)

Rizwan Ahmad, PhD, Senior Research Scientist
Advisor: PJ Utz
Department of Immunology & Rheumatology

Coexisting networks of TCRs and BCRs with mosaic public CDR3 sequences comprising conserved junctional motifs and their implications in immune responses and autoimmune disorders

Rizwan Ahmed, N. Majety, K.C. Chan, A. Giwa, H. Zhang, D.R. Bell, Y. Song, S. Lee, R. Zhou, R.M.Wolfe, T. Donner, C. Jie, A.R.A. Hamad

T cells and B cells constitute the adaptive immune system's formidable arms, orchestrating cellular and humoral immunity. These immune warriors wield intricate antigen receptor repertoires, with T cells employing the T cell receptor (TCR) and B cells brandishing the B cell receptor (BCR), also known as surface immunoglobulin. These receptors, pivotal for antigen recognition, harbor Complementary Determining Regions (CDR3) formed through somatic VDJ recombination and nucleotide alterations at V-D and D-J junctions. Challenging the traditional view of immune receptor diversity, we unveil two vast networks of TCRβ and IGH clonotypes, each originating from only two unique CDR3 sequences. Remarkably, they are associated with over 63 diseases. The TCRβ network features members

bearing the signature CDR3 sequence (CASSPGTEAFF), its N-terminal VD motif (CASSPGT) paired with various Jβ segments (CASSPGT-Jβx), or its DJβ motif recombined with various Vβ segments (Vβx-PGTEAFF). In contrast, the BCR network showcases one signature CDR3 sequence (CARx1-4DTAMVYYFYDW) derived from an invariant DJH motif (DTAMVYYFDYW) coupled with diverse VH genes. Strikingly, prototypes from both networks exhibit a teleological relationship, as they are co-expressed on rare dual expresser (DE) lymphocytes, with molecular dynamic simulations substantiating their interaction. We posit that these network members embody a core set of evolutionarily conserved primordial antigen receptors, playing pivotal roles in host defense and autoimmune diseases.

Gabe Barron, Immunology Graduate Student
Advisor: Mike Howitt
Department of Pathology, and of Microbiology & Immunology
The role of follicular-associated tuft cells on Peyer's patch dynamics

The intestinal mucosal barrier is tasked with discerning between friend and foe. These decisions are based a plethora of signals provided from the immune system, the epithelial barrier and the microbiome. In the small intestine, a majority of these immunological responses are initiated in tertiary lymphoid structures known as Peyer's patches. The hallmark function of the Peyer's patch is to process luminal antigens (food proteins, microbes, etc.) and mount antibody responses in the form of secretory IgA. These proteins are sampled via a specialized epithelial layer known as the follicular associated epithelium (FAE). An underappreciated player in Peyer's patch dynamics is the tuft cell, a chemosensory epithelial cell known for its ability to coordinate type-2 immune reactions in tissues across the body. Preliminary work shows that tuft cells are constitutively present at baseline amongst sampling microfold (M) cells and become a prominent feature of the FAE during a type-2 immune response in the intestine. This raises the central question of my project: how do FAE tuft cells augment the essential roles of the Peyer's patch? Thereby, my work centers tuft cells as a novel member of the FAE that influences intestinal IgA concentrations and specificities.

Leslie Chan, Immunology Graduate Student
Advisor: Catherine Blish
Department of Infectious Diseases
Functional and transcriptional investigation of NK responses in COVID-19 breakthrough infections

COVID-19 vaccines robustly protect against severe disease. As a higher proportion of the population becomes vaccinated against COVID-19, an increasing number of COVID-19 cases will occur in vaccinated individuals, i.e. "breakthrough" infections. Natural killer (NK) cells are uniquely poised to respond to SARS-CoV-2 variants capable of escaping antigen-specific responses, but little is known about the NK response during a breakthrough infection of

SARS-CoV-2. Additionally, adaptive-like NK cells are expanded in severe SARS-CoV-2 infection, but their functions in non-severe COVID-19 patients remain poorly understood. To investigate these questions, we compared adaptive-like and conventional NK cell function during COVID-19 delta infections in vaccinated versus non-vaccinated patients by utilizing mass cytometry. We further interrogated this via scRNA-seq and leveraged this dataset to identify correlates of NK responses in breakthrough infections and differences in NK cell-cell communication with other immune cell types in non-vaccinated vs. breakthrough patients. These findings will inform efforts to improve therapeutic and vaccination strategies for COVID-19, with therapeutic implications for other infectious diseases as well.

Izumi de los Rios Kobara, Immunology Graduate Student
Advisor: Catherine Blish
Department of Infectious Diseases

High natural killer cell proportion and immune activation correlate with low neutralization breadth in wild type SARS-CoV-2 infection

Natural Killer (NK) cells are underappreciated regulators of the antibody response to infection. In mice, NK cells can kill T follicular helper cells via perforin which decreases somatic hypermutation and vaccine response. The mechanisms of human NK cell regulation of antibody responses are not well understood, and it is not known whether NK cells influence the cross reactive response to emerging viral variants. In wild type (WT) SARS-CoV-2 infection, the antibody response is highly variable; some infected individuals develop neutralizing responses against distant variants whereas others fail to neutralize WT virus. We hypothesized that NK cells may regulate adaptive immune cells during SARS-CoV-2 infection and result in decreased antibody breadth against variants. Patients infected with WT SARS-CoV-2 from across the severity spectrum were profiled by single-cell RNA-sequencing, cyTOF, and neutralization breadth against variants of concern. We found that broad neutralizers had fewer NK cells that were less mature and proliferating. They also expressed Fas-L which may be more effective at targeting virus-infected cells without bystander killing of adaptive immune cells. NK cells from narrow neutralizers were highly activated and expressed gene signatures for viral infections as well as dysregulated inflammation. This work reveals that NK cell activation and dysregulated inflammation may play a role in poor antibody response to SARS-CoV-2 and opens exciting avenues for designing improved vaccines and adjuvants to target emerging pathogens.

Markus Diehl, Immunology Graduate Student
Advisors: Ed Engleman, Department of Pathology, and of Medicine - Immunology & Rheumatology and Nathan Reticker-Flynn, Department of Otolaryngology (Head and Neck Surgery

Neutrophil-activating therapy initiates systemic response to metastasis

Metastatic disease accounts for the majority of cancer-related deaths. The seeding and growth of metastases is often bolstered by the activity of neutrophils, which secrete pro-tumorigenic factors and suppress T cell responses. Conversely, neutrophils are also known to be critical responders to acute damage and infection. In order to harness the therapeutic potential of neutrophils, our lab developed a neutrophil-activating therapy (NAT) that consists of intratumorally administered anti-tumor antibody, anti-CD40 agonist antibody, and tumor necrosis factor (TNF). This therapy clears primary tumors in a T cell-independent manner and can also induce a T cell memory response to subsequent implantation. Furthermore, we have found that NAT reduces metastasis in several mouse models. I hypothesized that NAT induces a system-wide T cell-mediated response to metastasis that is dependent on activation of dendritic cells. Thus far, I have found that NAT is only able to reduce metastasis in T cell-sufficient mice. I will further elucidate the agents responsible for this anti-metastatic effect through cell-type specific depletion and rescue experiments. Furthermore, I will combine NAT with anti-PD-1 and CDK4/6 inhibitors to augment the response to metastasis. These experiments will advance our understanding of a new immunotherapeutic approach to cancer that has the potential to clear metastatic disease.

Xiaowen Ding, PhD, Postdoc
Advisor: Elizabeth Mellins
Department of Pediatrics - Human Gene Therapy

*Response of HLA-DRB1*15-restricted T cells in anakinra-associated delayed hypersensitivity reactions*

Systemic Juvenile Idiopathic Arthritis (SJIA), characterized by fever, evanescent rash, generalized lymphadenopathy and serositis, is a chronic inflammatory disease predominantly affecting pediatric populations. Improved outcomes have been observed with the increased use of IL-1/IL-6 neutralization therapies (e.g., anakinra (anti-IL-1) and tocilizumab (anti-IL-6)) post-2010. However, a subset of patients develops delayed hypersensitivity reactions that meet RegiSCAR criteria (1) for Drug Rash with eosinophilia and Systemic Symptoms (SJIA-DReSS). Risk of these drug-induced reactions is strongly linked to common HLA-DRB1*15 haplotypes, implicating CD4+ T cells. The mechanistic relationship of the drugs to the reaction remains to be elucidated. Here, we sought to determine the responses of HLA-DRB1*15-restricted CD4+ T cells after in vitro exposure to anakinra, as measured by the activation-induced marker assay. We found that anakinra can inhibit the activation of HLA-DRB1*15-restricted T cells induced by pooled peptides from human herpes viruses, such as EBV and HHV6, whereas anakinra alone was unable to stimulate T cells. Similar trends were also observed after replacing anakinra (drug preparation) with recombinant IL-1Ra. In addition, DRB1*15-binding anakinra peptides demonstrated reductions in the proportion of T cells activated by viral peptides. Thus, anakinra does not appear to function as an inciting antigen. Alternative hypotheses include that it suppresses T cell responses through blocking the co-stimulatory effect of IL-1 or that it interferes with the antigen presentation mediated by HLA-DRB1*15 molecules and consequently affects T cell responses.

Maïgane Diop, Immunology Graduate Student
Advisors: Catherine Blish, Department of Infectious Diseases and Brice Gaudillière, Department of Anesthesiology, Perioperative and Pain Medicine

Uterine natural killer cells modulate endometrial growth and persistence in endometriosis

Endometriosis is a chronic disease that affects 10% of reproductive-aged people with uteruses worldwide. It is characterized by growth of uterine endometrium in the peritoneum and marked by pelvic pain and infertility. There is currently no cure and treatments are limited in effectiveness. Recent work has revealed key changes in uterine NK Cells (uNK) in endometriosis as they dominate the uterine mucosa's immune landscape and play a crucial role in endometrial homeostasis. However, much of this work decouples uNK phenotypes and activity from uNK spatial interactions with endometrial innate immune and stromal cells. We hypothesized that aberrant uNK patterns of maturity, cytotoxicity, and receptor profile, coupled with spatial information on uNK interactions and organization could identify key features that could classify endometriosis. Here we present the largest endometriosis single-cell spatial dataset employing Imaging Mass Cytometry (IMC) analysis of eutopic endometrial biopsies from over 80 patients with stage I/II, stage III/IV, or no endometriosis for identification of key functional and spatial features that contribute to endometriosis. With IMC data at 1um resolution, we segment and phenotype immune and stromal cells in the endometrium for downstream analysis. This method allows us to couple previous single-cell knowledge of uNK cells in endometriosis with in situ spatial information, including neighborhood analysis and differential activity of stratum functionalis uNK cells and stratum basalis uNK cells. This work will inform understanding of uNK modulation of the uterine endometrium and innate immune cells therein, and the aberrations in this modulation that lead to endometriosis.

Tyson Holmes, PhD, Statistical Director
Human Immune Monitoring Center

Multiple regression methods for immunologists

Multiple regression is an essential statistical tool for the working immunologist. This presentation first defines multiple regression, then discusses availability and accessibility, provides some additional useful definitions, covers the topics of transformation and extreme value screening, and presents a scope and philosophy. Eleven different methods of multiple regression are then provided in detail, presented with strengths and limitations of each method. An emphasis is placed on application to immunological assays. Included is an easy-to-follow flowchart to guide immunologists' selection of multiple regression methods.

Shani Jahanbani, Research Associate
Advisor: Bill Robinson
Department of Immunology and Rheumatology
Increased macrophage phagocytic activity with TLR9 agonist conjugation of an anti- Borrelia burgdorferi monoclonal antibody

Shaghayegh Jahanbani, Paige S. Hansen, Lisa K. Blum, Effie E. Bastounis, Nitya S. Ramadoss, Mallesh Pandrala, Jessica Marie Kirschmann, Grace Sisemore Blacker, Zelda Z. Love, Irving L. Weissman, Fahimeh Nemati*, Michal Caspi Tal*, William H. Robinson*

Borrelia burgdorferi (*Bb*) infection causes Lyme disease, for which there is need for more effective therapies. Here, we sequenced the antibody repertoire of plasmablasts in *Bb*-infected humans. We expressed recombinant monoclonal antibodies (mAbs) representing the identified plasmablast clonal families, and identified their binding specificities. Our recombinant anti-*Bb* mAbs exhibit a range of activity in mediating macrophage phagocytosis of *Bb*. To determine if we could increase the macrophage phagocytosis-promoting activity of our anti-*Bb* mAbs, we generated a TLR9-agonist CpG-oligo-conjugated anti-BmpA mAb. We demonstrated that our CpG-conjugated anti-BmpA mAb exhibited increased peak *Bb* phagocytosis at 12–24 h, and sustained macro- phage phagocytosis over 60+ hrs. Further, our CpG-conjugated anti-BmpA mAbinduced macrophages to exhibit a sustained activation morphology. Our findings demonstrate the potential for TLR9-agonist CpG-oligo conju- gates to enhance mAb-mediated clearance of *Bb*, and this approach might also enhance the activity of other anti- microbial mAbs.

Madeline Lee, Immunology Graduate Student
Advisor: Catherine Blish
Department of Infectious Diseases
NK cell-monocyte crosstalk underlies NK cell activation in severe COVID-19

NK cells in the peripheral blood of severe COVID-19 patients take on a unique profile characterized by activation and dysfunction. Previous studies have identified soluble factors, including type I interferon and TGFβ, that underlie this dysregulation. However, the role of cell-cell interactions in mediating changes in NK cells during COVID-19 remains unclear. To address this question, we performed single-cell resolution interaction analysis through binning ("SCRIABIN") on existing single-cell RNA sequencing data to interrogate interactions between NK cells and other immune cells in severe COVID-19 patients. We found that NK cells interact most strongly with monocytes and that this occurs via both soluble factors and direct interactions. To validate these findings, we performed in vitro co-cultures in which NK cells from healthy donors were incubated with monocytes from COVID-19+ or healthy donors. Co-culture of healthy NK cells with monocytes from COVID-19 patients recapitulated aspects of the NK cell phenotype observed in severe COVID-19, including decreased expression of NKG2D, increased expression of activation markers, and increased proliferation. We also performed these co-cultures in a transwell system in order to isolate the effects of

soluble factors and profiled the cytokines present in these cultures. The results of these experiments suggest that both direct cell-cell interactions and cytokine signaling play a role in NK cell-monocyte crosstalk in COVID-19. Collectively, these results demonstrate that interactions between NK cells and monocytes in the peripheral blood of COVID-19 patients contribute to NK cell activation and dysfunction in severe COVID-19.

Claudia Macaubas, Research Scientist
Advisor: Elizabeth Mellins
Department of Pediatrics - Human Gene Therapy

Natural killer (NK) cells in patients with Pediatric Acute-onset Neuropsychiatric Syndrome (PANS)

Nelia Lechuga [1], Claudia Macaubas [1,2], Kerry Kizer [3], Danillo Augusto [3], Laurie Columbo [4,5], Cindy Manko [4,5], Abhinay Aeruva [4,5], Bahare Farhadian [4,5] Mellissa Silverman [4,6], Margo Thienenmann [4,6], Gonzalo Montera-Martin [7], Marcelo Fernandez-Vina [7], Catherine Blish [8] Jill Hollenbach [3], Jennifer Frankovich [4,5] & Elizabeth Mellins [1,2]

1. Department of Pediatrics, Stanford University
2. Program in Immunology, Stanford University
3. Department of Neurology, University of California San Francisco
4. Stanford Immune Behavioral Health Clinic and Research Program
5. Department of Pediatrics, Division of Allergy, Immunology & Rheumatology, Stanford
6. Department of Psychiatry, Division of Child & Adolescent Psychiatry, Stanford
7. Department of Pathology and Stanford Blood Center, Stanford University
8. Department of Medicine, Stanford University

Pediatric Acute-Onset Neuropsychiatric Syndrome (PANS) is characterized by the sudden onset of neuropsychiatric symptoms including obsessions/compulsions or food restrictions. PANS is commonly observed after infection (e.g., group A streptococcus or influenza), suggesting that an immune response, first triggered against infection, is likely involved in developing PANS. Natural Killer (NK) cells play a role in defense against infection, leading us to investigate a possible role of NK cells in PANS using both genetic and functional approaches. Analysis of HLA variation in 149 PANS cases and 2026 controls revealed a significant association of disease with the HLA-B locus, with the risk signal focused on positions 80-83. The amino acids at positions 80-83 comprise the Bw4 epitope, which mediates binding to the inhibitory receptor KIR3DL1 on the surface of NK cells. Of the four significant positions, HLA-B position 80-I (Isoleucine), which is known to increase binding affinity to KIR3DL1, displayed the strongest association ($p = 2.63E-05$, OR = 1.85, 95% CI = 1.36, 2.48). Next, we used previously frozen peripheral blood mononuclear cells (PBMC), and flow cytometry analysis to determine the NK cell number and marker expression in PANS patients at different disease stages and in healthy controls. We analyzed NK cells from 9 PANS patients at flare, 7 after improvement (7 pairs), and 9 healthy controls. The antibody panel included markers for NK cell subsets (CD56 and CD16), and NK receptors: KIR2DL1, CD158b (KIR2DL2/DL3), CD158e (KIR3DL1) and CD159a (NKG2A). These initial experiments suggest that activated, IFNγ+ CD56Bright and cytotoxic CD56Dim/CD16+ NK cells are more frequent among circulating immune cells in PANS patients compared to healthy controls. Also, there is a trend in both flare and improvement samples towards higher frequency of cells expressing CD158b (KIR2DL2/DL3) an inhibitory receptor that recognizes a subset of HLA-C alleles and lower frequency of cells that express CD159a (NKG2A), an inhibitory receptor that interacts with HLA-E molecules. Assessment of NK function through the CD107a degranulation assay are ongoing. Our results demonstrate inherited susceptibility to PANS linked to an HLA-B allele that regulates NK cells and suggest increased frequencies of circulating NK cells and of CD158b+ NK cells in PANS patients compared to healthy controls.

Evan Maestri, Immunology Graduate Student
Advisor: Purvesh Khatri
Department of (Research), Medicine, and of
(Research), Biomedical Data Science

Transcriptomic analysis of lupus reveals two disease endotypes

Background: Systemic lupus erythematosus (SLE) manifests as a heterogeneous autoimmune condition. It impacts multiple organ systems with a higher prevalence in women. In the last 50 years, more than 40 drugs have failed in clinical trials for lupus patients. The challenging nature of defining targeted-therapies for lupus highlights the major need for better molecular endotypes.

Methods: In this study, we integrated lupus single cell transcriptomics data from three cohorts including pediatric and adult patients totaling 1.64 million cells. Our work builds on a previous 93 gene-expression based signature for lupus (SLE MetaScore) defined in bulk transcriptome data. Here, we identify two lupus endotypes and examine their features in PBMC and whole blood microarray data comprising 22 datasets (662 Healthy, 4409 SLE).

Results: We identified that the SLE MetaScore is higher in all cell types for lupus patients compared to healthy, consistent with the systemic nature of disease. Next, we used hierarchical clustering to define two groups of lupus patients (high/low) based on their transcriptional expression of the SLE MetaScore. The high endotype exhibits a distinct set of proinflammatory CD14 and CD16 monocytes with elevated interferons. Additionally, we show the high group has reductions in the proportions of CD4 T cells, with an increased proportion of proliferating T/NK cells. Finally, the high endotype of lupus displays increased disease severity (SLEDAI), ds-dna autoantibodies, and reduced complement levels (C3, C4).

Conclusions: This work underscores the importance of improving the molecular characterization of lupus towards better disease monitoring and therapeutics.

Jae Seung Moon, PhD, Postdoc
Advisor: Bill Robinson
Department of Immunology and Rheumatology

Clonally Expanded Cytotoxic CD8+ T cells Target Citrullinated Antigens In ACPA+ Rheumatoid Arthritis

Jae Seung Moon[1,2], Shady Younis[1,2], Orr Sharpe[1,2], Navin L. Rao[3], Julie A. Carman[3], Eddie A James[4], Jane H Buckner[4], Kevin D. Deane[5], V Michael Holers[5], Laura T. Donlin[6], Mark M Davis[7], and William H Robinson[1]

[1]Stanford University School of Medicine, [2]VA Palo Alto Health Care System, [3]Janssen Research and Development, [4]Benaroya Research Institute, Translational Immunology, [5]University of Colorado Anschutz Medical Campus, [6]Hospital for Special Surgery, [7]Stanford University

The immune mechanisms that mediate synovitis and joint destruction in rheumatoid arthritis (RA) remain poorly defined. Although increased levels of CD8+ T cells have been described in RA, their role in pathogenesis remains unclear. Here we perform single cell transcriptome and T cell receptor (TCR) sequencing of CD8+ T cells derived from anti-citrullinated protein antibodies (ACPA)+ RA blood. We identify GZMB+CD8+ subpopulations containing large clonal lineage expansions that express cytotoxic and tissue homing transcriptional programs, while a GZMK+CD8+ memory subpopulation comprises of smaller clonal expansions that express effector T cell transcriptional programs. We demonstrate RA citrullinated autoantigens presented by MHC class I activate RA blood-derived GZMB+CD8+ T cells to expand, express cytotoxic mediators, and mediate killing of target cells. We also demonstrate that these clonally expanded GZMB+CD8+ cells are present in RA synovium. These findings suggest that cytotoxic CD8+ T cells targeting citrullinated antigens have a role in contributing to synovitis and joint tissue destruction in ACPA+ RA.

Jason Nideffer, Immunology Graduate Student
Advisors: Pras Jagannathan, Department of Medicine - Infectious Diseases, and of Microbiology & Immunology and Maria Grazia Roncarolo, Department of Pediatrics - Stem Cell Transplantation, and of Medicine - Blood & Marrow Transplantation

The pediatric CD4+ T cell response to malaria

At the center of immunity, CD4+ T cells provide antigen-specific instructions to innate phagocytes, cytotoxic killers, and antibody-secreting plasma cells. However, relatively little is known about the transcriptional programs or clonality of these cells in the context of malaria. We applied single-cell genomics to longitudinally study memory CD4+ T cell responses to repeated natural infections in Ugandan children. Briefly, memory CD4+ T cells were isolated from PBMC samples following a brief ex vivo rest or stimulation with Plasmodium-infected red blood cells and then subjected to droplet-based single-cell RNA/TCR sequencing. From these data, we consistently observed clonal expansion of Plasmodium antigen-specific T cells following malaria—a response that was dominated by type I regulatory T (Tr1) cells expressing high levels of IL10, LAG3, and several co-inhibitory receptors. Clonal expansion of these Tr1 cells peaked shortly after malaria, but contraction happened slowly, and Tr1 effectors continued to express IL10 for multiple months following antimalarial drug administration. Finally, Tr1 clones demonstrated long-term memory and phenotypic stability, expanding and producing IL10 in response to repeated infections. By identifying malaria-specific memory subsets and tracking T cell clonal dynamics through multiple infections, we contribute important insights into the quality, dynamics, and fidelity of memory CD4+ T cell responses to natural Plasmodium infections.

Trung Pham, MD, PhD, Assistant Professor
Department of Pediatrics – Infectious Diseases
Uncovering mechanisms of tissue immunity and immunophysiology during persistent infection

The immune system safeguards the health of complex organisms by rapidly eliminating invading pathogens and maintaining tissue homeostasis. Many bacterial pathogens, such as Salmonella enterica and Mycobacterium tuberculosis, evade host antimicrobial mechanisms and persist in infected tissues for months and years even in the presence innate and adaptive immune resistance. Macrophages are heterogenous mononuclear phagocytes that exist in all mammalian tissues and act as critical sensors and regulators of tissue homeostasis. Macrophages can also kill bacteria, coordinate immune responses to eliminate pathogens, and paradoxically, act as a cellular niche that enables intracellular bacteria to persist in infected tissues within granulomas, which are immunological structures formed to contain infection and comprised of diverse immune cell types. We seek to define the development, maintenance, and plasticity of macrophage functional diversity and mechanisms of tissue repair and nutrient regulation during persistent infection. We employ a murine Salmonella Typhimurium infection model and bring together immunology, tissue biology, microbiology, and genetics to uncover mechanisms underlying tissue immunity and immunophysiology from the molecular to organismal level. Here, we apply single-cell transcriptomics to define critical pathways and cellular crosstalk underlying macrophage functional diversity and tissue maintenance during persistent splenic infection. We describe a regulatory circuit that involves the transcriptional factor SPIC, which controls macrophage heme metabolite sensing and recycling functional program, gene targets of SPIC, and macrophage-T cell crosstalk. Preliminary data are presented that show the SPIC pathway facilitates persistent infection. These findings suggest that mechanisms controlling heme homeostasis may impact pathogen survival in infected tissues.

Ruoxi Pi, PhD, Postdoc
Advisor: Catherine Blish
Department of Infectious Diseases
Engineering natural killer (NK) Cells to target Human Immunodeficiency Virus (HIV) reservoirs

HIV is a retrovirus that integrates its proviral genome into host cells. The infected cells that escape immunosurveillance persist to become latent reservoirs, which poses the main barrier to a cure. HIV reservoirs have been identified in multiple tissues and cell subsets, necessitating targeting and elimination of these reservoirs to achieve a HIV cure. One potential mechanism for this elimination is through natural killer (NK) cells, innate lymphocytes that efficiently eliminate tumor and virus-infected cells through cytotoxicity. Enhanced NK cell activity has been detected in individuals who resist infection despite high levels of exposure (so called highly exposed

seronegative individuals) and in individuals with a delayed progression to AIDS, suggesting that NK cells have the intrinsic ability to target HIV-infected cells. In addition, compared to cytotoxic CD8 T cells, allogeneic NK cells can be transferred with little risk of graft-versus-host disease (GvHD), and therefore hold great promise as an 'off-the-shelf' product for immune cell therapy. Here, we investigate multiple strategies to enhance the ability of NK cells to target HIV reservoirs. We engineer NK cells with chimeric NK cell receptors, which bind to ligands that are upregulated in HIV-infected cells and can induce robust activation signaling through their intracellular domains. We also engineer NK cells to overexpress chemokine receptors that facilitate lymphocyte homing into different compartments of secondary lymphoid tissues to target CD4 T cell subsets that have been identified as HIV reservoirs. Our work will inform the design of adoptive NK cell therapeutic strategies for HIV cure efforts.

Andrea Reitsma, Lab Manager/Clinical Research Coordinator and
Ayantika Sen, PhD, Postdoc
Advisors: Sheri Krams and Olivia Martinez
Department of Surgery – Abdominal Transplantation

Longitudinal immune profiling of whole blood reveals sustained expansion of γδ T cells in children with multisystem inflammatory syndrome

Andrea Reitsma*, Ayantika Sen*, Jing Guo#, Anika Shah*, Mahil Rao*, Yueh-hsiu Chien#, Sheri Krams*, Olivia Martinez*
*Transplant Immunology, Department of Surgery, Stanford University
#Department of Microbiology and Immunology, Stanford University

Multisystem inflammatory syndrome in children (MIS-C) is a late onset, but severe hyperinflammatory condition associated with SARS-CoV-2 infection. As part of the Pediatric Research Immune Network on SARS-CoV-2 and MIS-C (PRISM) trial, 244 pediatric patients (0-21 years), diagnosed with either MIS-C or COVID, were enrolled to determine the immune and clinical features underlying MIS-C. We analyzed blood samples, collected from 58 subjects (25 COVID and 33 MIS-C), at baseline prior to treatment, at 28 days, 6 months, and 12 months post-diagnosis. Whole blood from these subjects were analyzed using the Maxpar Direct ImmunoProfiling Assay (MDIPA) tube that includes a 30-marker antibody panel, followed by mass cytometry and data analysis using Pathsetter software to identify 37 immune cell subsets. As previously reported, granulocyte subsets in MIS-C patients were dysregulated, with increased neutrophils (p=0.0007) observed. Analysis of lymphoid and myeloid populations revealed a significant increase in proportions of total B cells (p=0.0002), $CD4^+$ T cells (p=0.0193) and non-classical monocytes ($CD11c^+HLA\text{-}DR^+CD14^-CD38^-$) (p=0.0026) in the MIS-C group compared to the COVID group. However, proportions of total T cells (p=0.0145), dendritic cells (p=0.0001), NKT/MAITs ($CD3^+CD161^+CD28^+$) (p=0.0258) and classical monocytes($CD11c^+HLA\text{-}DR^+CD14^+CD38^+$) (p=0.0162) were significantly decreased in MIS-C patients compared to COVID patients. By 28 days post-diagnosis the proportions of these immune cell subsets in MIS-C patients had returned to levels similar to COVID patients at the same time point and remained unchanged for at least six months. Interestingly, the proportion of γδ T cellsin MIS-C patients were comparable to the COVID group at baseline but were significantly elevated in MIS-C at 28 days (p=0.0068) and 6 months (p=0.0058). We further analyzed the γδ T cells using

CATALYST and CITRUS algorithms and identified four subclusters of gd T cells that were predominantly T effector memory phenotypes that were dysregulated in MIS-C patients. Our findings indicate that MIS-C is characterized by generalized immune dysregulation that resolves within 28 days. Interestingly, however a longitudinal expansion of circulating γδ T cells is observed in children with MIS-C.

Sarah Sackey, Immunology Graduate Student
Advisor: Catherine Blish
Department of Infectious Diseases
Establishing tonsil and splenic organoids as a model of HIV infection in secondary lymphoid tissues

Currently, 39 million people are living with human immunodeficiency virus (HIV). HIV is a lifelong infection due to the viral DNA inserting itself into the host CD4 T cells' genome where it can lay dormant, a state known as latency. These latently infected cells are long-lived and challenging to eradicate. They can also re-activate at any time to produce more virus, driving the infection and preventing a cure. CD4+ T follicular helper (Tfh) cells residing in secondary lymphoid organs (SLOs) such as the tonsil and spleen harbor the majority of the latent HIV reservoir. Thus to study the latently infected cells in SLO, we harnessed the tonsil and splenic organoid system developed by the Davis Lab which recapitulates the 3D architecture and functions of these SLOs. We were able to successfully infect the tonsil and splenic organoids and confirmed Tfh and memory CD4+ T cell populations as the most highly infected CD4+ T cell population confirming results found in other previous work. Overall these studies demonstrate our ability to successfully infect these organoids with HIV and provide proof of concept to further develop them as a model of HIV latency in SLOs.Our goal is to develop this platform to mimic the in vivo cellular architecture and complexity of latent HIV infection and to test our therapeutic approaches to eliminate the latent HIV reservoir in patients living with HIV.

Aiswarya Sethumadhavan, PhD, Postdoc
Advisor: Eric Meffre
Department of Immunology & Rheumatology
Assessment of the tolerogenic role of B cells during pregnancy

B cells have been suggested to play a role during pregnancy in the establishment of tolerance at the fetal-maternal interface. However, transgenic mouse models have shown that a two-fold increase in estrogen or prolactin favors the production of autoreactive B cells. To determine the impact of the elevated concentrations of reproductive hormones during pregnancy on the removal of developing autoreactive B cells, we analyzed the reactivity of recombinant antibodies cloned from single transitional and mature naïve B cells isolated from women during their first, second and third trimester of pregnancy and several months postpartum. Our preliminary data suggest that central B cell

tolerance is abrogated during pregnancy and results in the production of many autoreactive transitional B cells. In addition, we found that new emigrant/transitional B cells preferentially accumulate in the decidua basalis at the maternal interface with the placenta in which they account for about a quarter of all decidual B cells. In contrast, we found that the frequency of autoreactive mature naïve B cells remained low in the peripheral blood of pregnant women, we therefore propose that the broadening of the BCR repertoire during pregnancy will favor the presentation of fetal antigens by decidual B cells to autoreactive T cells and convert them into regulatory T cells via IL-10 production to ensure tolerance at the fetal-maternal interface. Understanding the tolerogenic role of B cells during pregnancy may help us to propose novel therapeutic strategies to prevent reproductive complications in which B cells are suspected to be involved.

Vishnu Shankar, Immunology Graduate Student
Advisors: Mark Davis, Department of Microbiology & Immunology and Paul Mischel, Department of Pathology

Using single-cell RNA sequencing data on human patient biopsies to define new therapeutic targets in follicular lymphoma

Vishnu Shankar[1], Sarah Haebe[2,4], Tanaya Shree [3,4], Ronald Levy[4]

1 Program in Immunology, Stanford University School of Medicine, Stanford, CA, USA;
2 Department of Medicine III, LMU Hospital Munich, Munich, Germany;
3 Division of Hematology & Medical Oncology, Oregon Health & Sciences University, Portland, OR, USA;
4 Division of Oncology, Stanford University School of Medicine, Stanford, CA, USA;

Follicular lymphoma (FL), one of the most common types of indolent non-Hodgkin's lymphoma, is characterized by abnormal B cell growth in the lymph node follicle. As 20% of FL patients relapse in the first two years following treatment initiation due to insufficient removal of cancer cells and therapeutic resistance, there is a need for identifying new molecular targets, which can guide the development of novel therapies. To identify candidate tumor-specific targets, we used single-cell RNA sequencing data across 50,102 total cells from fine needle aspirates (FNA) from 12 patients to compare the gene expression profiles of FL tumor and normal B cells. FL tumor B cells were designated by monoclonal light chain expression. To identify important transcriptomic differences between FL and normal B cells, lasso regression techniques were used to select 298 differentially expressed genes from 18,780 total expressed coding genes. This model achieved 93.2% cross-validation accuracy and 97.4% validation accuracy on 102,625 total cells from 17 patients in distinguishing normal and tumor transcriptomic profiles. As the genes up-regulated in FL are more likely to be drug targets, we focused our analysis accordingly. Among the 72 genes up-regulated in FL, our unbiased approach captured known molecular targets of FL, including BCL2, the anti-apoptotic gene that critically drives FL pathogenesis. Further analysis of up-regulated genes converged on several dysregulated pathways in FL, including nutrient uptake, glycolysis, leukotriene metabolism, calcium signaling, and cytoskeleton regulation. Experiments are planned to validate protein level differences and identify which protein targets may correspond to "druggable" opportunities.

Sasa Vasilijic, PhD, Senior Research Scientist Basic Life Scientist
Advisor: Konstantina Stankovic
Department of Otolaryngology (Head and Neck Surgery)
Identification of immune-related candidate biomarkers in plasma of patients with sporadic vestibular schwannoma

Vestibular schwannoma (VS) is an intracranial tumor arising from neoplastic Schwann cells, and typically presenting with hearing loss. The traditional belief that hearing deficit is caused by physical expansion of the VS, compressing the auditory nerve, does not explain the common clinical finding that patients with small tumors can have profound hearing loss, suggesting that tumor-secreted factors could influence hearing ability in VS patients. We conducted profiling of patients' plasma for 66 immune-related factors in patients with sporadic VS (N>170) and identified and validated candidate biomarkers associated with tumor size (S100B) and hearing (MCP-3). We further identified a 9-biomarker panel (TNR-R2, MIF, CD30, MCP-3, IL-2R, BLC, TWEAK, eotaxin, S100B) with outstanding discriminatory ability for VS. These findings revealed possible therapeutic targets for VS, providing a unique diagnostic tool that may predict hearing change and tumor growth in VS patients, and may inform the timing of tumor resection to preserve hearing.

Clarence Rachel Villanueva, MS, Life Science Research Professional
Advisor: Eric Meffre
Department of Medicine - Immunology & Rheumatology
Anti-B cell therapy may prevent the production of autoreactive B cells in relapsing remitting multiple sclerosis

B-cell depleting monoclonal antibodies have proven to be extremely potent in reducing or stopping disease activity in relapsing remitting multiple sclerosis (rrMS). The long-term effect of anti-B cell therapy in rrMS contrasts with its short-term impact on other autoimmune diseases and may correlate with the specific pattern of B-cell tolerance defect in rrMS compared to other autoimmune diseases. Indeed, a large proportion of patients with rrMS display a proper removal of developing autoreactive B cells in the bone marrow, whereas this central B cell tolerance checkpoint is impaired in all other autoimmune diseases. To determine the impact of short-term anti-B cell therapy in rrMS on autoreactive B cell selection, we analyzed the frequencies of autoreactive new emigrant/transitional and mature naïve B cells before and after two courses of ocreluzimab anti-CD20 antibody injection in nine patients with rrMS. We found that patients who relapsed displayed an impaired central B cell tolerance, whereas patients who did not relapse showed low frequencies of autoreactive B cells exiting the bone marrow. In addition, anti-B cell therapy restored the impaired autoreactive B cell selection in the periphery of the patients who displayed a functional central B cell tolerance but not in those with defective central B cell tolerance.

Hence, rrMS is a heterogeneous disease that can be stratified into two distinct entities based on specific pattern of B-cell tolerance defect and the efficacy of B-cell depleting antibodies may be linked to a normal central B-cell tolerance and the production of normal B-cell and T-cell compartments.

Edward Vizcarra, PhD, Postdoc
Advisor: Olivia Martinez
Department of Surgery - Abdominal Transplantation

Characterization of EBV-specific peripheral T cells from pediatric transplant patients with elevated EBV DNAemia or controlled infection

Edward A. Vizcarra, Irene Liang, Carlos O. Esquivel, Sheri M. Krams, Olivia M. Martinez

The gamma-herpes virus, Epstein-Barr Virus (EBV), is one of the most prolific viruses in the world infecting approximately 90-95% of the adult human population. EBV infection is typically asymptomatic in most individuals but can also lead to self-limiting infectious mononucleosis. Following immune-mediated control, the virus persists for the lifetime of the host in a quiescent state. Pro-inflammatory T cells are the predominant mediators controlling EBV following primary infection and viral reactivation in the previously infected. However, EBV is also linked to a variety of epithelial and lymphoid malignancies, and immunocompromised individuals are at a particular risk for the development of EBV-associated malignancies. Moreover, some transplant patients placed on immuno-suppressive medications that target the T cell response consequently develop EBV disease such as uncontrolled viremia and B cell lymphomas termed posttransplant lymphoproliferative disease (PTLD). However, it is unclear what immune characteristics are required for protective immunity of EBV, or those signatures that lead to a lack of viral control. We hypothesize that individuals who cannot control EBV infection are deficient in a T cell profile that promotes an efficient anti-viral response. To test this hypothesis, peptides derived from latent cycle EBV antigens wereused to stimulate PBMCs from pediatric transplant patients that are EBV controllers(n=11) and those with chronic EBV DNAemia (n=11). 24 hours post-stimulation, we performed scRNA-sequencing to characterize the gene expression and T cell receptor profiles. In parallel stimulated cultures, multi-parameter flow cytometry was used to determine T cell activation, memory status, and effector function. We postulate that patients with high EBV viral load will lack an adequate memory repertoire marked bysuboptimal levels of IFNγ or immune exhaustion. Our long-term goal is to define the key attributes that are required for protective immunity against EBV, and to identify immune biomarkers for individuals who may be susceptible to the development of EBV-associated cancers.

Oliver Wirz, PhD, Postdoc
Advisor: Scott Boyd
Department of Pathology

Systematic multi-antigen analysis of SARS-CoV-2-specific B cells defines distinct memory and functional subsets in blood and lymphoid tissues of patients and vaccinees

Oliver Wirz[1,§], Fan Yang[1,§], Molly Hunter[2], Emily Haraguchi[1], Maxim Zaslavsky[1,3], Erin Craig[4], Mark M. Davis[5], Bali Pulendran[1], Kari Nadeau[6], Sharon Chinthrajah[6], Jeremy Minshull[2], Claus Niemann[7,8], Scott D. Boyd[1,6]

[1] Department of Pathology, Stanford University, Stanford, CA
[2] ATUM Bio, Newark, CA
[3] Department of Computer Science, Stanford University, Stanford, CA
[4] Department of Biomedical Data Science, Stanford University, Stanford, CA
[5] Stanford Institute for Immunity, Transplant and Infection, Stanford University, Stanford, CA
[6] Sean N. Parker Center for Allergy and Asthma Research, Stanford University, Stanford, CA
[7] Department of Anesthesia & Perioperative Care, University of California, San Francisco, CA
[8] Department of Surgery, University of California, San Francisco, CA
[§] These authors contributed equally

Following SARS-CoV-2 infection or vaccination, B cells mount a protective immune response binding viral antigens with their surface B cell receptor (BCR) and secreting virus-specific antibodies. There is still incomplete understanding of the extent to which vaccine- and infection-induced B cell responses differ at a clonal level. In addition, tissue-localized B cell responses to SARS-CoV-2 remain understudied. Here, we isolated antigen-specific B cells using large panels of DNA- and fluorophore-tagged Wuhan-Hu-1 and viral variant proteins. Sorted SARS-CoV-2-specific B cells from blood of infected patients and mRNA vaccine (BNT162b2) recipients analyzed by single cell sequencing identified the specific B cell receptors and the antigens and variants bound to these cells. Our highly multiplexed panel of DNA-tagged antigens included full SARS-CoV-2 Spike, S1, S2, receptor binding domain (RBD) including 7 or 20 viral variant RBDs, and nucleoprotein (N), to analyze the BCR specificity of over 4500 antigen-binding B cells from 17 vaccinees and 28 infected patients at different timepoints after SARS-CoV2 vaccination or infection, and spleen, lymph nodes, blood and bone marrow of 21 deceased organ donors. Our data indicate differences in B cell phenotypes as well as BCR antigen-binding breadth and sequence composition in responses to infection compared to vaccination.

Stanford MEDICINE Immunology

School of Medicine

THANK YOU FOR ATTENDING!

Keep an eye out for the next Stanford Immunology Scientific Conference.