Stanford

IMMUNOLOGY POSTDOCTORAL SYMPOSIUM 2023

Conference organized by
Stanford Immunology Postdoc Committee

Discover new areas of research, connect with future collaborators, and expand your network in the research community.

EVENT DETAILS:

APRIL 26, 2023 | 8:30 AM – 5:30 PM
CHEM-H JOHN A. AND CYNTHIA FRY
GUNN ROTUNDA, E241
STANFORD, CA 94305

IMMUNOLOGY POSTDOCTORAL SYMPOSIUM 2023

Welcome to the 2023 Stanford Immunology Postdoc Symposium!

We are excited to welcome postdocs, visiting fellows, scholars, and everyone to a day featuring amazing keynote speakers and panel discussions highlighting and celebrating work from early career researchers and industry experts.

This one-day in-person event is held at the at Chem-H John A. and Cynthia Fry Rotunda, followed by a reception and poster presentation at the Chem-H Courtyard. The symposium provides opportunities for networking and discussions.

Attendees will have the opportunity to discover new areas of research, connect with future collaborators, and expand your network in the research community. We welcome you!

Stanford MEDICINE Immunology
School of Medicine

AGENDA AT A GLANCE

TIME	TOPIC
8:30-9:00 AM	Breakfast
9:00-9:15 AM	**Opening**: Olivia Martinez, PhD, Professor of Surgery | Director of Graduate Program, Immunology IDP | Director of Stanford Immunology
9:15-10:15 AM	**Keynote**: Christopher Barnes, PhD, Assistant Professor of Biology
10:15-10:25 AM	Break
10:25-11:15 AM	**Session 1**: Pablo Domizi, PhD, Instructor of Pediatrics
11:15AM-12:55 PM	Lunch and Poster Session
1:00-2:00 PM	**Keynote**: Christina Curtis, PhD, MSc, Professor of Medicine, Genetics and Biomedical Data Science
2:00-2:45 PM	**Session 2**: Postdoc talks
2:00-2:15 PM	Nick Chamberlain, Postdoctoral Scholar, Eric Meffre Lab
2:15-2:30 PM	Ru Wen, PhD, Postdoctoral Scholar, James Brooks Lab
2:30-2:45 PM	Colwyn (Coco) Headley, PhD, Postdoctoral Scholar, Phillip Tsao Lab
3:00-4:00 PM	**Panel Discussion**: Nima Aghaeepour, PhD, Sean Bendall, PhD, Berenice Mbiribindi, PhD, and Varnesh Tiku, PhD
4:00-4:15 PM	**Vote of thanks**: Betsy Mellins, MD, Professor of Pediatrics, Chair of Stanford Immunology Postdoctoral Committee
4:15-5:30 PM	**Social/Reception**

The Immunology Postdoc Symposium 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.

2023 Immunology Postdoc Committee
Thank you to the symposium planning committee and your dedication on making the event a success.

Director: Elizabeth Mellins
Members: Yudi Bedi, Sayane Shome, Manoj Kumar, Ayantika Sen, Abhishek Koladiya, Surbhi Sharma, and Tatjana Bilich
Staff: Torye Nguyen and Michele King

KEYNOTE SPEAKERS

We are honored to have two Keynote Speakers:

Christopher Barnes, PhD
Assistant Professor of Biology at Stanford
www.thebarneslab.com/

Christina Curtis, PhD, MSc
Professor of Medicine, Genetics and Biomedical Data Science at Stanford
www.med.stanford.edu/curtislab

Christopher Barnes, PhD is an Assistant Professor of Biology and Sarafan ChEM-H Institute Scholar whose research leverages interdisciplinary approaches to address fundamental principles of viral-host interactions for therapeutic benefit. Before arriving at Stanford, Dr. Barnes earned degrees in Psychology (BA) and Chemistry (BS, MA) from the University of North Carolina at Chapel Hill (G. Pielak), and completed his Ph.D. thesis at the University of Pittsburgh (G. Calero). Following this training, he completed postdoctoral research at the California Institute of Technology, where he combined biophysical methods with in vivo approaches to understand how viruses such as HIV-1 and SARS-CoV-2 infect host cells and elicit specific humoral immune responses (P. Bjorkman). Over the course of the COVID-19 pandemic, he has made significant contributions to our understanding of antibody-spike interactions through in-depth structural analysis that detail the specificities and mechanisms of how monoclonal neutralizing antibodies bind spike to prevent infection. His work in structure-guided approaches to the treatment of infectious disease has earned him several awards, including recognition as a Rita Allen Foundation Scholar, an HHMI Hanna H. Gray Fellow, and appointment as a Chan Zuckerberg Biohub investigator. Now, the Barnes laboratory investigates viral-host interactions and translates knowledge of the structural correlates of antibody-mediated neutralization of viruses into the rational development of highly protective antibodies. The long-term goal of this work will be structure-based design of potent and stable immunogens for vaccination against emerging and re-emerging zoonotic viruses.

Christina Curtis, PhD, MSc is an Endowed Professor of Medicine and Genetics at Stanford University where she leads the Cancer Computational and Systems Biology group. Dr. Curtis also serves as the Director of Breast Cancer Translational Research and Co-Director of the Molecular Tumor Board at the Stanford Cancer Institute. Dr. Curtis's laboratory leverages computational modeling, high-throughput molecular profiling and experimentation to develop new ways to prevent, diagnose and treat cancer. Her research has helped to redefine the molecular map of breast cancer and led to new paradigms in understanding how human tumors evolve and metastasize.

Dr. Curtis is the recipient of numerous awards, including those from the V Foundation for Cancer Research, STOP Cancer and the American Association for Cancer Research (AACR). She received the National Institutes of Health Director's Pioneer Award in 2018, the Stanford Prize in Population Genetics and Society (2020) and was named an In vivo Rising Leader in the Life Sciences (2021) and the Julius B. Kahn Visiting Professor in the Dept of Pharmacology, at Northwestern University (2020). In 2022 she received the AACR Award for Outstanding Achievement in Basic Science. Dr. Curtis is also Kavli Fellow of the National Academy of Sciences, a Susan G. Komen Scholar and a Chan Zuckerberg Biohub Investigator.

Dr. Curtis serves as a scientific advisor to multiple academic institutes and biotech and was elected to the AACR Board of Directors in 2022. She also serves on the editorial board of journals spanning computational biology to precision oncology.

SESSION 1

Pablo Domizi, PhD
Instructor, Pediatrics – Hematology & Oncology at Stanford University

Pablo Domizi, PhD is an Instructor of Pediatrics in the Division of Hematology and Oncology at Stanford School of Medicine, working with Dr Kara Davis. Dr Domizi holds a PhD in molecular biology from National University of Rosario, Argentina. In 2018, Dr. Domizi joined Dr. Kara Davis' lab as a postdoc, where he gained expertise in single cell technologies and B cell development. Currently, his scientific interests combine transcriptional regulation and antigen modulation in B cell malignancies. His research has unraveled new roles for IKAROS in CD19 modulation and CART19 failure, identifying it as a potential prognostic target.

SESSION 2

Nick Chamberlain, Postdoctoral Scholar, Eric Meffre Lab

Human TLR7 deficiency is associated with the production of autoreactive B cells

Nicolas Chamberlain, Natsuko Yamakawa, Henner Morbach, Yile Dai, Sally Yraita, Fatma Naz Cemre Kalayci, Salomé Glauzy, Jean-Nicolas Schickel, Bertrand Boisson, Raul Jimenez-Heredia, Aaron M. Ring, Megan A. Cooper, Anthony Hayward, Charlotte Cunningham-Rundles, Kaan Boztug, Caspar I. Van der Made, Alexander Hoischen, Klaus Warnatz, Jean Laurent Casanova, and Eric Meffre

Mouse models revealed a key role for TLR7 in promoting autoimmunity. Similarly in humans, a gain-of-function TLR7 mutation has been identified in a patient with systemic lupus erythematosus (SLE), whereas TLR7 deficiency was reported to confer severe COVID-19 susceptibility. We have identified four patients with distinct TLR7 missense mutations associated with the development of autoimmune hemolytic anemia and other non-SLE autoimmune manifestations. Surprisingly, these TLR7 mutations were loss-of-function mutations that altered TLR7 conformation and resulted in a failure to activate plasmacytoid dendritic cells, monocytes, and B cells following TLR7 stimulation. To evaluate the impact of TLR7 deficiency on the establishment of B cell tolerance, we analyzed the reactivity of recombinant antibodies cloned from single B cells isolated from TLR7-deficient patients with or without autoimmune manifestation and compared them with counterparts from mother carriers and related non-carriers. We found elevated frequencies of autoreactive mature naïve B cells in all TLR7-deficient patients but not in related family members. However, Rapid Extracellular Antigen Profiling (REAP) analysis revealed that TLR7-deficient patients did not display serum autoantibodies besides one who showed exacerbated TLR8 function likely due to the inability of his mutated TLR7 to bind UNC93B-1. We also found increased serum IFNλ2 concentration and decreased T cell expression of SLAM-associated protein (SAP) in TLR7-deficient patients, both of which may contribute to the impaired peripheral tolerance checkpoint in TLR7-deficient patients. Hence, TLR7 deficiency results in the production of autoreactive B cells that may favor the development of autoimmunity, and potentially also promote poor COVID-19 outcome.

Ru Wen, PhD, Postdoctoral Scholar, James Brooks Lab

Siglec-7/9 are novel immune checkpoints for prostate cancer

Ru M Wen, Jessica C Stark, G. Edward W Marti, Fernando García-Marqués, Hongjuan Zhao, Rosie Nolley, Carolyn R Bertozzi, Sharon J Pitteri, James D Brooks

Immune checkpoint-based therapy has led to striking improvements in survival in some types of cancer, but it is ineffective for others, including prostate cancer. Changes in glycosylation, particularly hypersialylation, have been found in diverse malignancies. Glycosylation can result in immune evasion through direct interactions of sialylated glycoproteins on cancer cell surfaces with Siglecs on immune cells including T cells, NK cells, and dendritic cells. Interactions between Siglec-7/9 and sialic acid have been implicated in inhibiting immune response in melanoma, leukemia, and lung cancer. Here, we found that Siglec-7/9 was highly expressed in immune cells including T cells and myeloid cells in the prostate cancer TCGA dataset. Immunohistochemistry demonstrated that Siglec-7/9 ligands were highly expressed in surgically resected prostate cancer tumor tissues but showed no or low expression in adjacent normal tissues. Siglec-7/9 ligands were also found in the prostate cancer cell lines. The disruption of Siglec-7/9 and their ligands by sialidase, which cleaves the sialic acid, or by anti-Siglec-7/9 blocking antibodies, promoted T cell-mediated cytotoxic killing of prostate cancer cells. Furthermore, anti-Siglec-7/9 treatment suppressed prostate cancer tumor growth in a humanized mouse model. Immunohistochemistry demonstrated that prostate cancers in mice treated with anti-Siglec 7/9 antibodies exhibited less Ki67 and CD31, and more cleaved caspase 3 and CD8 compared with the isotype control. This study provides insight into Siglec-7/9-sialic acid targeting strategies for prostate cancer and lays the groundwork for developing a novel class of immunotherapy-based drugs.

Colwyn Headley, PhD, Postdoctoral Scholar, Phillip Tsao Lab

Mitochondrial transplantation rescues aging-associated T cell dysfunction

Colwyn A. Headley, Shalini Gautam, Angelica Olmo-Fontanez, Andreu Garcia-Vilanova, Varun Dwivedi, Anwari Akhter, Alyssa Schami, Kevin Chiem, Russell Ault, Hao Zhang, Hong Cai, Alison Whigham, Jennifer Delgado, Amberlee Hicks, Philip S. Tsao, Jonathan Gelfond, Luis Martinez-Sobrido, Yufeng Wang, Jordi B. Torrelles & Joanne Turner

Mitochondrial dysfunction alters cellular metabolism, increases oxidative stress, and may be principal to the dysregulated signaling and function of CD4+ T lymphocytes in the elderly. Mitochondria influence adaptive immune responses through glucose and fatty acid metabolism, calcium buffering, and redox signaling. By transferring young mitochondria into CD4+ T cells from old mice, we investigated whether aging-associated mitochondrial dysfunction could be abrogated and whether such transfer changed CD4+ T cell function. Our results show that mitochondrial transfer improved the redox status and function of CD4+ T cells from old mice ex vivo, and in vivo mito-transferred CD4+ T cells protected Rag1-KO mice from viral and non-viral pathogens. These findings support the notion that mitochondria can serve as targets of therapeutic intervention in aging.

PANEL DISCUSSION

Nima Aghaeepour, PhD
Associate Professor of Anesthesiology, Perioperative and Pain Medicine, and of Pediatrics at Stanford
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Sean Bendall, PhD
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Berenice Mbiribindi, PhD
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Varnesh Tiku, PhD
Scientist at Gilead
www.linkedin.com/in/varneshtiku/

Dr. Nima Aghaeepour is an Associate Professor at Stanford University. Our laboratory develops machine learning and artificial intelligence methods to study clinical and biological modalities in translational settings. He primarily focuses on leveraging multiomics studies, wearable devices, and electronic health records to address global health challenges. Prior to my his as a faculty member, he studied for my B.Sc. in computer science from University of Tehran, followed by a PhD in Bioinformatics from the University of British Columbia and a postdoctoral fellowship at Stanford.

Dr. Aghaeepour is an alumnus of the Graduate School of Business's Ignite program, and a Biodesign Faculty Fellow, and a SPARK fellow. He also regularly serves on the scientific advisory boards of a wide range of companies, and plays with a stealth-mode startup or two. He strongly encourages (and funds) his trainees to take advantage of Stanford's unique entrepreneurship training programs. He believes the next generation of successful academic life scientists will be both multidisciplinary and entrepreneurial.

He is a pilot (for small/acrobatic aircraft) and a wingsuiter. He also regularly plays the piano. His other interests include swimming, mountain climbing, chess, Starcraft, target shooting, modern art, and Mandarin Chinese.

Dr. Sean Bendall: Our goal is to understand the mechanisms regulating the development of human systems (both embryonic and adult). In particular, we are interested in clarifying the roles of both protein coding genes as well as pathobiology (disease state or pathogen) known to be uniquely human – therefore, not analogously studied in model organisms. Drawing on both pluripotent stem cell biology, hematopoiesis, and immunology, combined with novel high-content single-cell analysis (CyTOF Mass Cytometry) and imaging (MIBI Multiplexed Ion Beam Imaging) we are creating templates of 'normal' human cellular behavior. Using these we can decipher the roles of protein regulators on cellular specification as well as the influence of human-specific pathobiology on system remodeling at the single cell level. This work will enable a better understanding of how disease corrupts this process. Ultimately, our objective will be to use such approaches to not only reveal how novel regulators function in the context of complex cellular systems, but also enable the mechanistic characterization of human pathobiology in primary human tissues. In doing so we will understand how changes in related physiological or pathological systems can be more readily recognized and controlled.

In addition to the lab's work on human hematopoiesis and pluripotent stem cell specification we are seeking collaborative partnerships surrounding problems in human immunology as well as in regenerative medicine, including efforts to exploit next generation single-cell analysis and new computational methods to create systems level models of these processes so that they may be better understood and directed.

Dr. Berenice Mbiribindi is a Principal Scientific Researcher at Genentech in the department of Translational Oncology focusing on Cancer Immunotherapy. She leads Biology studies for drug discovery projects focused on immune cell engaging antibody therapies (artificial immunity). Her work involves close collaboration and study coordination with other Research labs including Antibody Engineering, Biochemical Pharmacology, Proteomics, Bioinformatics, Safety/Toxicology, and other core drug discovery groups.

Before joining Genentech, Dr. Mbiribindi was a Postdoctoral Research Fellow at Stanford University – school of Medicine (Transplant Immunology Lab). Combining in silico and in vitro analysis, she was working on understanding the role of NK cells in the context of Epstein Barr

Virus (EBV) latent infection and their involvement in EBV related complications. She received the Transplant and Tissue engineering Center of Excellence leadership group Fellowship and the Stanford Maternal and Child Health Research Institute Fellowship. Additionally, she has been awarded The Transplantation Society Young Scientific Investigator Award in 2018 for her work.

Beside her research, Dr. Mbiribindi did mentor several students in the lab and she is an active member in the Black In Immuno organization.

Dr. Varnesh Tiku is currently working at Gilead Sciences as a scientist in immuno-oncology employing multiple approaches to discover novel drug targets to boost T cell immunity against tumors. Before joining Gilead Sciences, Dr. Tiku was a scientist at Vir Biotechnology where he led efforts on discovering novel druggable host targets to limit bacterial and viral infections in humans using functional genomics approaches.

Originally from India, he did his master's from Skövde University Sweden and Max Planck Institute of Molecular Cell Biology and Genetics in Dresden Germany. His PhD work involved investigating the role of the nucleolus in the regulation of aging and immunity at Max Planck Institute for Biology of Ageing in Cologne Germany. After his PhD, he joined Genentech as a postdoctoral researcher and studied the pathogenic mechanisms employed by bacteria like A. baumannii and P. aeruginosa in their infection cycle.

POSTER SESSION

Inna Averbukh, PhD, Mike Angelo Lab

Spatiotemporal coordination at the maternal-fetal interface promotes trophoblast invasion and vascular remodeling in the first half of human pregnancy

Shirley Greenbaum, Inna Averbukh, Erin Soon, Gabrielle Rizzuto, Alex Baranski, Noah F. Greenwald, Adam Kagel, Marc Bosse, Eleni G. Jaswa, Zumana Khair, Shirley Kwok, Shiri Warshawsky, Hadeesha Piyadasa , Mako Goldston, Angie Spence, Geneva Miller, Morgan Schwartz, Will Graf, David Van Valen, Virginia D. Winn, Travis Hollmann, Leeat Keren, Matt van de Rijn, Michael Angelo

Beginning in the first trimester, fetally-derived extravillous trophoblasts (EVTs) invade the uterus and remodel its spiral arteries, transforming them into large, dilated blood vessels. Several mechanisms have been proposed to explain how EVTs coordinate with the maternal decidua to promote a tissue microenvironment conducive to spiral artery remodeling (SAR). However, it remains a matter of debate which immune and stromal cells participate in these interactions and how this evolves with respect to gestational age. Here, we used a multiomic approach combining the strengths of spatial proteomics and transcriptomics to construct the first spatiotemporal atlas of the human maternal-fetal interface in the first half of pregnancy. We used multiplexed ion beam imaging by time-of-flight (MIBI-TOF) and a 37-plex antibody panel to analyze ~500,000 cells and 588 arteries within intact decidua from 66 patients between 6-20 weeks of gestation, integrating this with coregistered transcriptomic profiles. Gestational age substantially influenced the frequency of maternal immune and stromal cells, with tolerogenic subsets expressing CD206, CD163, TIM-3, Galectin-9, and IDO-1 increasingly enriched and colocalized at later time points. In contrast, SAR progression preferentially correlated with EVT invasion and was transcriptionally defined by 78 gene ontology pathways exhibiting unique monotonic and biphasic trends. Lastly, we developed an integrated model of SAR where invasion is accompanied by upregulation of pro-angiogenic, immunoregulatory EVT programs that promote interactions with vascular endothelium while avoiding activation of maternal immune cells.

Maya Baron, PhD, Julien Sage Lab

T cells in SCLC: tilting the balance towards anti-cancer effects

Maya Baron, Debadrita Bhattacharya, Alexandros Drainas, Julien Sage.

Small cell lung cancer (SCLC) is an aggressive neuroendocrine cancer associated with heavy tobacco smoking. The combination of late detection and limited therapeutic options results in a dismal overall 5-year survival of ~6%. In recent years, immune checkpoint inhibitors activating T cells have emerged as a new therapy to treat SCLC. However, only a small fraction of SCLC patients benefit from these immunotherapies. Single-cell RNA sequencing analysis of T cells in SCLC tumor microenvironment revealed that only some T cells express cytotoxic genes, while most T cells express exhausted and immunosuppressive gene signatures. These observations raised the question if SCLC-associated T cells possess cytotoxic functions. Using a series of functional assays in genetically engineered mouse models of SCLC, we uncover an unexpected function of a subpopulation of CD3+ T cells isolated from

SCLC tumors that significantly promotes SCLC growth by inhibiting apoptosis of cancer cells. Comparing Bulk RNA sequencing of tumors where T cells were present or absent uncovered that T cells induce the expression of the survival receptor CD74 in SCLC cells. Examination of CD74's role in SCLC revealed that CD74 is essential for SCLC survival, and inhibition of CD74 expression significantly reduces SCLC cell viability and disease progression. Our work aims to provide novel insights into the pro-survival mechanism underlying the resistant nature of SCLC to current treatments and suggest that targeting CD74 signaling in SCLC may serve as a novel therapeutic strategy for SCLC patients.

Yuhan Bi, Eugene Butcher Lab

Why are HEV high? Role of the Ire1-Xbp1 pathway in the morphology and function of high endothelial venues

Yuhan Bi, Kevin Brulois, Julian pan, Eugene Butcher.

High endothelial venules (HEV) are specialized portals for lymphocyte entry into lymphoid tissues and sites of chronic inflammation from the blood. They regulate immune cell trafficking in physiologic and pathologic settings including autoimmune diseases, inflammation, and cancer. HEV are characterized by dramatically expanded endoplasmic reticulum (ER), Golgi and secretory machinery, features responsible for their 'high' (plumb, cuboidal) morphology; but neither the mechanism driving this defining phenotype, nor its functional significance are known. Leveraging a scRNAseq atlas of endothelial cells from lymph nodes, we find that Xbp1 and Ern1 (encoding the enzyme IRE1) and genes associated with the unfolded protein response (UPR), are selectively elevated in venules and especially in HEV. The IRE1α-XBP1 axis mediates ER expansion in 'professional secretory cells'' such as antibody producing plasma cells. The UPR counteracts "endoplasmic reticulum (ER) stress". We show that HEV in mice treated with an IRE1 inhibitor, or in mice with endothelial-specific deletion of Xbp1, lose their 'high' endothelial phenotype. Electron microscopy reveals altered ER and Golgi associated with thinning and with apoptotic changes. While the vessels retain significant peripheral node addressin (PNAd) expression, Xbp1 deletion significantly reduces lymphocyte homing into lymph nodes. Together these findings show that the Xbp1 pathway drives the unique morphology of HEV, and that the 'highness' of HEV is a direct consequence of mechanisms that support HEV survival and function in lymphocyte recruitment.

Hansen Chen, PhD, Gary Steinberg Lab

Rapid complement activation induced by acute hyperglycemia worsens blood-brain barrier leakage and ischemic stroke outcome

Hansen Chen, Terrance Chiang, Anika Kim, Stephen Tomlinson, Tonya M. Bliss*, Michelle Y. Cheng*, Gary K. Steinberg*

Background: Acute hyperglycemia, which occurs in over 40% of ischemic stroke patients regardless of pre-existing diabetes, increases brain swelling, hemorrhagic transformation (HT) and worsens stroke outcome. Understanding the mechanisms of hyperglycemia-exacerbated stroke injury will be vital for developing novel treatments. Here we identify systemic complement activation as a novel contributor to hyperglycemic-exacerbated damage in rodent stroke.

Method: Male C57/BL6 mice (10-11 weeks) were subjected to sutured induced-middle cerebral artery occlusion (MCAO) for 30 min, the suture was then removed to restore cerebral blood flow, simulating thrombectomy-induced recanalization in clinical stroke. Acute hyperglycemia was induced by glucose injection 10 min before MCAO. To assess the effect of hyperglycemia on stroke outcomes, mice were sacrificed at 4.5 and 24 hr post-stroke to analyze brain swelling, blood-brain barrier (BBB) leakage, and HT; or allowed to survive 14 days to examine mortality rate, neurological deficit, and motor-sensory dysfunction using the horizontal rotating beam test. Complement activation was evaluated at 1, 2, 4.5 hr post-stroke. To investigate the role of C3 in hyperglycemic-exacerbation of stroke pathology we inhibited C3 activation, using the targeted complement inhibitor CR2-Crry fusion protein, or PBS control, injected intraperitoneally 30 min after reperfusion; we also assessed the motor function of C3 knockout mice following stroke.

Result: Hyperglycemia worsens stroke outcomes in this cerebral ischemia-reperfusion model, with rapidly increased BBB leakage ($p<0.0001$), brain swelling ($p<0.05$) and HT ($p<0.0001$) at 4.5 and 24 hr after stroke, when compared to normoglycemia. Hyperglycemia stroke mice also exhibit poorer outcome including higher mortality (100% vs 25%, $p=0.0008$), body weight loss, and impaired behavioral performance at the sub-acute phase. Notably, acute hyperglycemia rapidly increased plasma complement C3 level at 1 and 2 hr after stroke ($p<0.01$), accompanied by rapid and time-dependent activation of C3 in ischemic brain vessels as indicated by increased vascular C3d. Additionally, activated C3d levels colocalized with IgM/IgG and positively correlated with brain swelling and HT ($p<0.01$). Pharmacological inhibition of C3 by CR2-Crry significantly reduced brain C3d($p<0.05$), IgM ($p<0.05$), and IgG levels ($p<0.01$) at 4.5 hr and improved neurological deficits at 24 hr ($p<0.01$). Furthermore, C3 knockout mice were also protected from the detrimental effects of acute hyperglycemia on stroke exhibiting significantly lower brain levels of IgM ($p<0.05$) and IgG ($p<0.01$), with improved neurological deficit scores ($p<0.01$) and motor function.

Conclusion: Rapid activation of complement C3 by acute hyperglycemia is an important contributor to BBB leakage, brain swelling and HT after experimental stroke. Inhibiting C3 activation could be a potential therapeutic approach to improve stroke outcomes, particularly for those with hyperglycemia. Our study also opens the door to explore the role of systemic C3 in other pathologies with increased BBB leakage such as aging.

Acknowledgment: This study is supported by grants NIH Grant R01NS064136C and NINDS R01NS093057 (GKS); AHA postdoc fellowship 916011 (HC). We thank Dr. Katrin Andreasson and Dr. Louise D. McCullough for scientific discussion.

Ivana Cvijovic, Steve Quake Lab

The statistics of tissue residence and migration in the human B cell repertoire

Ivana Cvijovic*, Michael Swift*, Stephen Quake (*equal contribution, ordered alphabetically)

B cells generate pathogen-specific antibodies, providing adaptive protection against infectious microbes. Unlike most proteins, antibodies are not genetically encoded at birth but are stochastically generated and modified in evolutionary processes acting on B cell populations within an individual. Although these populations expand, migrate, and home to long-term niches throughout the body, the relationships between B cell populations and the connections between differentiation, proliferation signals, and migration patterns remain unclear. To address this, we sequenced the B cell receptors

(BCRs) and transcriptomes at the single-cell level in multiple immune-rich tissues from six individuals, enabling us to examine the patterns of expansion, migration, and differentiation of related B cells. While most BCRs appear only in single tissues, we observed B cells with identical BCRs in multiple tissues. These cells, often linked with recent expansion, are transcriptionally distinct from other populations and exhibit more hypermutated BCRs than cells confined to a single tissue. Employing phylogenetic approaches, we discovered that related B cell groups (lineages) tend to co-localize within the same tissue. However, when lineages reach a threshold size in the peripheral blood and secondary lymphatic organs (SLOs), their members are more likely to be found in other tissues. This indicates that cellular migration from blood and SLOs is a probabilistic sharing process, which occurs when lineages attain sufficient size. Furthermore, we identified a hierarchy in migration patterns between tissues, with the spleen as the primary destination for hypermutated B cells and the bone marrow as the final location. Notably, we observed a marked distinction between all examined tissues and the bone marrow, which had the largest fraction of private lineages, consistent with its role as a terminal destination for differentiated B cells. Collectively, our findings elucidate the evolutionary landscape of B cell migration across tissues and suggest that the peripheral blood provides limited insights into the dynamics and composition of the human B cell repertoire.

Antonio Delgado Gonzalez, PhD, Garry Nolan Lab

High-grade serous ovarian tumor cells modulate NK cell function to create an immune-tolerant microenvironment

Antonio Delgado-Gonzalez, Ying-Wen Huang, Veronica D. Gonzalez, Kenyi Donoso, Shih-Yu Chen, Karen Sachs, Andrew J. Gentles, Grace M. Allard, Kevin S. Kolahi, Brooke E.Howitt, Ermelinda Porpiglia, Garry Nolan, Wendy J. Fantl

Tubo-ovarian high-grade serous carcinoma (HGSC) is a lethal gynecologic malignancy with an overall 5-year survival rate of ~50%. For the past 30 years, standard-of-care has been surgical debulking combined with platinum-based chemotherapy. Most women initially respond to this therapy, but ~80% eventually relapse within 3-years. Multi-parameter single-cell technologies have recently revealed a high intra- and inter-cellular heterogeneity in HGSC, which is in part related to treatment failure. A mechanistic understanding of the molecular and cellular events that promote heterogeneity and lead to HGSC relapse is of utmost importance. We characterized intra-tumoral T and natural killer (NK) cells in newly diagnosed HGSC by single-cell mass cytometry (CyTOF). We identified intra-tumoral decidual-like (dl)-NK cells (CD56+CD9+CXCR3+KIR+CD3-CD16-), which have an immune tolerant role in preventing a mother-to-be from rejecting her hemi-allogenic fetus, that were positively correlated with the abundance of tumor cells and transitioning epithelial-mesenchymal cells. We showed combinatorial expression patterns of ligands for activating and inhibitory NK receptors within three HGSC tumor compartments; epithelial (E), transitioning epithelial-mesenchymal (EV) and mesenchymal (vimentin-expressing (V)), and observed a striking inhibitory ligand signature in V cells. In in vitro cocultures, NK cells acquired CD9 from HGSC tumor cells by trogocytosis, resulting in reduced anti-tumor cytokine production and cytotoxicity. Cytotoxicity in these cocultures was restored by treatment with a CD9 blocking antibody and CD9 knockout performed using CRISPR/Cas9. Our results identify trogocytosis of CD9 as a previously unrecognized mechanism of immune suppression in HGSC, and CD9 as a promising new therapeutic target with immediate relevance for HGSC immunotherapy.

Qiwen Deng, PhD, Gerlinde Wernig Lab

uncovering the spatial transcriptomic signature of diabetic nephropathy

Diabetic nephropathy (DN) is a prevalent cause of chronic kidney disease worldwide, resulting in glomerular hypertrophy, glomerulosclerosis, and tubulointerstitial inflammation and fibrosis. Despite current therapies, no specific treatment is available to prevent or reverse renal fibrosis in DN. Identifying novel drug targets that can halt the progression of DN and prevent end-stage kidney disease, which is a significant contributor to morbidity and mortality.

Single-cell sequencing has proven to be a valuable tool in characterizing the transcriptomic signature of various cell types within the kidney. However, DN is a disease with a regional spatial distribution that heterogeneously affects cells. In this study, we characterized the spatial transcriptomic signature of DN patients using VISIUM and Molecular Cartography. By combining this with single-cell sequencing data, we were able to deconvolute the signature of each spatial transcriptomic spot, mapping 20 dominant cell types to human diabetic nephropathy and identifying the patterns of colocalization between innate immune cells and fibroblasts.

We subsequently used CO-Detection by indEXing (CODEX) to capture gene signatures underlying this colocalization. Interestingly, we identified a novel biomarker, CD63, which is highly expressed in fibroblasts in patients with renal fibrosis in DN. This finding indicates that CD63 is a crucial determinant in the development of renal fibrosis in DN, as fibroblasts are the key source of pathological extracellular matrix (ECM) deposition, leading to disruption of tissue architecture and organ dysfunction. Our study provides valuable insights into the pathogenesis of renal fibrosis in DN and identifies potential drug targets for halting its progression.

Denis Dermadi, PhD, Purvesh Khatri Lab

Systems biology of chromatin states and histone crosstalk in the human immune system

Denis Dermadi, Laurynas Kalesinskas, Ananthakrishnan Ganesan, Alex Kuo, Peggie Cheung, Sarah Cheng, Mei Dvorak, Thomas J. Scriba, Aida Habtezion, Michele Donato, Paul J. Utz, Purvesh Khatri

Chromatin remodeling through post-translational modifications of histone tails, or histone modifications, plays a crucial role in regulating and maintaining DNA-centered processes. However, due to technical challenges, the systems-level coordination and interactions between histone modifications and their impact on the functional state of immune cells have remained largely unexplored.

To address this, we utilized advanced computational analysis and a large, biologically heterogeneous dataset of over 27 million cells from 158 healthy human donors. We profiled primary human immune cells for 33 histone modifications and 4 histone variants at the single-cell level using high-dimensional mass cytometry (EpiTOF). This enabled us to map relationships between histone modifications at the systems level and discover highly conserved chromatin states associated with critical DNA-centric processes, including gene transcription, apoptosis, and regulation, across different immune cell types.

We developed a comprehensive epigenetic network of histone modification associations using a computational interpretation framework based on neural processes (NP). This enabled us to learn the

directional networks of associations between histone modifications, recapitulating known associations and predicting novel ones.

In a separate cohort of healthy subjects vaccinated with the trivalent inactivated seasonal influenza vaccine (TIV), we applied our models and identified changes in associations between six pairs of histone modifications 30 days following vaccination, many of which are functionally involved in innate memory.
Overall, our study provides a resource foundation for future research aimed at understanding the complexity of histone modification interactions in immune responses in infectious or autoimmune diseases, cancers, and vaccination.

Jessica L. Fessler, PhD, Justin Sonnenburg Lab

Mining the gut microbiome of cancer patients to identify microbes associated with response to immunotherapy

Jessica Fessler, Matthew R. Olm, Justin L. Sonnenburg

Cancer immunotherapy has revolutionized oncology, but despite its success, the benefits are limited to a minority of patients. Among the variables linked to outcome, the composition of the gut microbiome has emerged as one feature associated with efficacy. While revelatory, consistent, and well-defined links between the microbiome and response remain obscure, likely due to small sample sizes and variable methods. To overcome these limitations and identify robust connections, we conducted a comprehensive meta-analysis using high resolution metagenome-based de novo genome assembly profiling methods incorporating 12 studies representing 800+ cancer patients. Top taxa associated with response were then evaluated in preclinical mouse models to evaluate function.

We found significant differences in both the alpha and beta diversity between studies. To account for inter-study differences, we used a logistic regression model to identify taxa associated with outcome. Abundance of the bacteria Barnesiella intestinihominis was among those positively associated with response to immunotherapy. Immune-modulatory potential of B. intestinihominis was evaluated using a mouse melanoma model, B16.F10-OVA, treated with immunotherapy, anti-PD-L1. Consistent with patient data, mice colonized by a human isolate of B. intestinihominis had better tumor control, characterized by heightened intra-tumoral T cell proliferation and expansion of tumor-specific CD8+ T cells. Colonization by B. intestinihominis was marked by robust induction of Th17 cells in the intestinal lamina propria, as well as increased proliferation in all T cell compartments. Further studies will refine the mechanism of action for B. intestinihominis and test additional microbes that can be exploited to improve immunotherapy outcomes.

Dorien Feyaerts, PhD, Brice Gaudillière Lab

Cross-tissue assessment of local and peripheral immune determinants of preeclampsia

Dorien Feyaerts, Joshua Gillard, Ina A. Stelzer, Ivana Marić, Julien Hédou, Maïgane Diop, Edward A. Ganio, Xiaoyuan Han, Kazuo Ando, Amy S. Tsai, Thomas A. Bonham, Masaki Sato, Jakob Einhaus, Max Sabayev, Nima Aghaeepour, Martin S. Angst, Roberto Romero, Mark Hlatky, David K. Stevenson, Virginia D. Winn, Nardhy Gomez-Lopez, Brice Gaudillière

Preeclampsia (PE) is a severe pregnancy complication that affects 3-5% of all pregnancies worldwide and significantly impacts maternal as well as child mortality and morbidity, and yet, remains unpredictable. Dysregulations of pregnancy-induced immune adaptions are increasingly implicated in PE pathogenesis and provide a promising strategy to identify predictive factors of PE before onset of clinical symptoms. In this study, up to 11 blood samples were collected per participant at monthly intervals (gestational week 12-40; N = 225) for longitudinal analysis of immune trajectories (suspension mass cytometry) and plasma proteomics to differentiate normotensive (n=14) and preeclamptic (n=7) pregnancies. Paired placental tissue (FFPE) was collected at delivery for local immune landscape and spatial organization analysis of the feto-maternal interface using imaging mass cytometry. Longitudinal analysis showed shared (e.g., DDR1) and differentiating (e.g., Siglec-6) plasma proteomic trajectories in control compared to PE-affected pregnancies. In addition, increased CD44 expression by circulating T cell subsets over gestation of PE-affected pregnancies suggests an activated and antigen-experienced adaptive immune response. Furthermore, increased CPT1a expression in T cells at the start of the 3th trimester implicates increased fatty acid oxidation utilization during PE-affected pregnancies. The results provide the basis for ongoing studies integrating the dynamic assessment of systemic immune responses differentiating PE from normotensive pregnancies with high-plex imaging of the local immune microenvironment for a cross-tissue characterization of PE immune pathogenesis. Given the global impact of PE, this work is a key step towards both defining mechanistic targets derived from blood-based early PE diagnosis and guiding potential therapeutic interventions for PE, thereby improving mother and child health.

Michael Freedman, MD, MPhil, Purvesh Khatri Lab

Identification of conserved detrimental host immune response predicts severity of bacterial and viral infections

Michael Freedman, Hong Zheng, Denis Dermadi, Aditya Rao, Laurynas Kalesinskas, Lara Murphy Jones, Benjamin Solomon, Purvesh Khatri

Host immune response has been repeatedly shown to diagnose the presence and type of infection. Recently, we described a 42-gene blood-based signature, conserved across viruses, that correlates with and predicts the severity of viral infection, irrespective of age, sex, and host or pathogen genetics. This gene signature is composed of 4 modules (2 protective, 2 detrimental). We hypothesize that these modules are also associated with disease severity in patients with bacterial infection. We analyzed 30 publicly available datasets of blood transcriptome profiles from 3312 patients (1033 healthy, 1085 with bacterial infection, 1194 with viral infection). Severity was stratified into seven categories ranging from healthy to fatal. We evaluated our four module and composite severe-or-mild “SoM” scores, and applied our previously described 7-gene signature to both bacterial and viral samples. Similar to viral infections, two detrimental module scores were positively correlated with severity of bacterial infections (module 1: r=0.64, module 2: r=0.53), and one of two protective modules was inversely correlated (module 4: r=-0.59). Module 3, protective in viral infections, was minimally positively correlated with severity of bacterial illness (module 3: r=0.20). SoM score was positively correlated with severity (r=0.63) and distinguishes non-severe from severe bacterial infections (AUROC=0.74). SoM score can distinguish patients with severe infection, irrespective of bacterial or viral etiology. When used in conjunction with our 7GS, it may help decide whether a patient should be (1) treated with an antibiotic and (2) discharged or admitted to hospital upon presentation to an emergency department.

Sanjana Gupta, PhD, Purvesh Khatri Lab

A nine-gene blood-based signature meets the WHO TPPs for diagnosis of active tuberculosis and predicting progression from latent to active disease.

Aditya Rao, Sanjana Gupta, Madeleine Scott, Valeriu Crudu, Timothy Rodwell, Donald Catanzaro, Antonino Catanzaro, Purvesh Khatri

As part of its End TB strategy, the WHO has identified the need for non-sputum-based diagnostics that meet target product profiles (TPP) of 90% sensitivity and 70% specificity for diagnosis of ATB and 75% sensitivity and specificity for predicting progression from LTB to ATB. The successful translation of a 3-gene blood-based signature, identified using diverse datasets, into a prototype point-of-care diagnostic, that meets the WHO TPPs, has demonstrated the power of integrating large amounts of heterogeneous data to identify generalizable disease signatures. We hypothesized that integration of more diverse datasets, comprising patients with ATB or other inflammatory lung diseases (e.g., COPD, viral infections, sarcoidosis, lung cancer, etc.), would identify novel robust signatures, for diagnosing ATB and predicting progression from LTB to ATB, that meet the WHO TPPs.

By integrating data from 3615 peripheral blood samples across 49 publicly available transcriptomic datasets, we identified a 9-gene signature for diagnosing ATB patients from healthy controls, or individuals with LTB or other diseases. The signature achieved 90% sensitivity and 81% specificity in retrospective validation cohorts (3836 blood samples, 28 datasets) and 90% sensitivity and 70% specificity in a prospective cohort from Moldova (360 blood samples). In a longitudinal cohort of adolescents, the 9-gene signature predicted progression from LTB to ATB up to 1 year prior to sputum conversion with 76% sensitivity and 75% specificity. specificity. Finally, the signature predicted prolonged lung inflammation in the Catalysis Treatment Response Cohort. Overall, the 9-gene signature meets the WHO TPPs required for the End TB strategy.

Mathangi Janakiraman, PhD, Laren Becker Lab

Fermented food improves gut motility and alters MM phenotype

Primary hypothesis: We posit that FF dietary supplementation can reverse age-dependent gut dysbiosis and restore gut function to geriatric mice by modulating host immunity. Aim: Characterizing the effects of FF on the gut microbiota, gut functionality, and the gut immune system in geriatric mice, and identifying potential therapeutic interventions for chronic inflammation in aging. Methods: Old (19-24 months old) WT C57BL/6 mice were given water supplemented with a sterile fermented brine drink (16.7% v/v) for 2 months. We assessed gut motility by measuring whole gut transit times and bead expulsion (colonic transit) times. We also evaluated gut barrier integrity by proxy using measures of LPS levels in serum using a TLR4 reporter cell line and albumin levels in feces. Further, we used flow cytometry, FACS, ELISA and qPCR to characterize the MMs. We are also in the process of characterizing the gut microbiota through 16S rRNA sequencing of fecal DNA. Results: We observed that FF supplementation in old WT C57BL/6 mice resulted in improved colonic motility – as evidenced by shorter colonic transit times, with no change in whole gut transit times. But gut barrier integrity was unaffected. While there was no change in the proportions of various immune cells, we also observed increased expression of Hcar2 (a butyrate sensing GPCR with known anti-inflammatory role) and IL-10

by the colonic MMs in mice given FF compared to mice on normal drinking water. Further plans: We are analyzing other geriatric, inflammatory and homeostatic genes in the MMs, and trying to identify how Hcar2 may influence gut functionality and inflammation. Parallelly we are characterizing the gut microbiota alterations through 16S rRNA sequencing, to eventually associate that with functional and immune changes. Successful completion of the study will deliver a mechanistic understanding of how fermented foods affect gut homeostasis with aging. Our results will provide proof-of-principle that dietary intervention can be a successful strategy for maintaining a healthy gut microbiota and youthful gut function.

Adrien Mirouse, Eric Meffre Lab

PTPN22 inhibition prevents the production of autoreactive B cells in systemic lupus erythematosus

Adrien Mirouse, Fatma Naz Cemre Kalayci, Yann Le Guen, Esen Sefik, Warren D. Reynolds, Fabien R. Delmotte, Jeff W. Chen, Richard Flavell, and Eric Meffre

Objective: While anti-B cell therapies show efficacy in the treatment of autoimmunity, we aimed at exploring an alternative therapeutical strategy that would specifically prevent autoreactive B-cell production by resetting defective early B-cell tolerance checkpoints in patients with autoimmune diseases. Since a PTPN22 loss-of-function polymorphism was reported to protect against systemic lupus erythematosus (SLE), our objective was to assess the effect of PTPN22 inhibition on autoreactive B-cell production.

Methods: MISTRG6 humanized mice were generated after allograft with hematopoietic stem cells (HSCs) harvested from 3 healthy donors (HD) and 5 patients with SLE. Mice were then subjected to LTV-1 or A1907001 PTPN22 inhibitor or control DMSO injections twice daily for one week. Recombinant antibodies were cloned from single transitional B-cells isolated from both humanized mice and subjects and their reactivity tested by ELISA and immunofluorescence assays. scRNA analysis was performed using bone marrow enriched CD19+ B cell precursors.

Results: Autoreactive B cell frequencies were elevated (36%) in MISTRG6 mice engrafted with HSCs isolated from SLE patients and similar to counterparts in patient's blood (24%). In contrast, PTPN22 blockade significantly decreased autoreactive B cell proportions that averaged 8% ($p<0.0001$) and were similar to those from MISTRG6 mice transplanted with HSCs from HD. scRNA sequencing analysis revealed that PTPN22 inhibition reduced interferon-signature and strengthened BCR signaling.

Conclusion: PTPN22 inhibition restore impaired central B-cell central tolerance in SLE and may thwart autoimmunity via the development of a normal B cell compartment that no longer supports the production of pathogenic autoantibodies or autoimmune manifestation.

Ruoxi Pi, PhD, Catherine Blish Lab

Engineering natural killer (NK) cells to target human immunodeficiency virus (HIV) reservoirs

Ruoxi Pi, Nancy Zhao, Sarah Sackey, Marion Santo, Elsa Solà, Mark Davis, Catherine Blish

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.

Stephan Ramos, PhD, Seung Kim Lab

Optimized bone marrow conditioning and tolerance assays to advance cell- based therapies for diabetes

Stephan A. Ramos, Preksha Bhagchandani, Qizhi Tang, Audrey V. Parent, Everett Meyer, Seung K. Kim

While significant advances have been made in exogenous insulin delivery and glucose monitoring for the treatment of diabetes, these approaches are not curative. Islet transplantation is a current and attractive alternative to insulin replacement therapy, but several challenges remain before broader adoption of this option, especially in children. These include the prevention of recurrent autoimmunity and the achievement of islet graft tolerance without islet-toxic systemic immunosuppression. Mixed hematopoietic cell chimerism after hematopoietic cell transplant (HCT) is a viable option, if 'reduced intensity' bone marrow conditioning methods were developed to reduce or eliminate X-ray irradiation (XRT). Additionally, the development of human HSC-based mechanisms for achieving islet allotolerance or autoimmune suppression would benefit from novel experimental systems to model human allo- and auto-tolerance. We systematically evaluated the synergistic effect of the clinically relevant JAK/STAT inhibitor, baricitinib, and decreased amounts of low dose total body irradiation in conjunction with our preestablished monoclonal antibody targeting of c-Kit, and T cell depleting antibodies conditioning regime on the generation of mixed hematopoietic chimerism across fully mis-matched major histocompatibility complex (MHC) barriers in immunocompetent mice. Here, we have achieved durable mixed-hematopoietic chimerism with as low as 75cGy TBI. Further work will investigate whether the addition of other monoclonal antibodies to the conditioning regime results in a further reduction in the amount of TBI required to achieve mixed-hematopoietic chimerism. This work could then be applied to generate novel in vivo models of human mixed-hematopoietic chimerism to investigate the mechanisms of human islet allo-/auto-tolerance and autoimmunity.

Masaki Sato, MD, PhD, Brice Gaudillière Lab

A multiplex mass cytometry assay for high-throughput immunomodulatory assessment of candidate drugs to prevent preterm birth

Masaki Sato, Jakob Einhaus, Dorien Feyaerts, Joshua Gillard, Maximilian Sabayev, Edward A. Ganio, Ina A. Stelzer, Julien Hedou, Kazuo Ando, Amy S. Tsai, Adam Bonham, Maigane Diop, Dyani K. Gaudilliere, Ivana Maric, Martin Angst, Gary Shaw, Nima Aghaeepour, Garry Nolan, Linda Giudice, Jean Costello, Tomiko Oskotsky, Marina Sirota, David Stevenson, Brice Gaudilliere

Preterm birth (PTB) is the world's leading cause of mortality in children under the age of five. While multiple biological and exogenous factors contribute to PTB, increasing evidence suggests that dysfunctional maternal immune adaptations play a key role in the pathogenesis of PTB. Therefore, targeting the mother's immune system is a promising approach to the prevention of PTB. Few immunomodulatory therapies have been tested in this context, as significant concerns exist for potential off-target and harmful effects.

Here, we screened preselected, pregnancy-safe compounds, previously identified by a transcriptomics-based computational drug repositioning pipeline (Le et al. 2020), for their immunomodulatory capacity and potential to reverse preterm-birth profiles.

Using a high-throughput multiplex mass cytometry screening assay, over 1500 intracellular signaling responses were simultaneously assessed in 25 innate and adaptive immune cell subsets in peripheral whole blood across a range of drug concentrations and stimulation conditions. Out of 13 candidate drugs, lansoprazole, iopamidol, and cefotaxime showed the strongest modulation of signaling activity changes induced by stimulation. When matching notable cell-type and pathway-specific immunomodulatory effects with gestational immune profiles, pravastatin, rifubatin, and clotrimazole showed the highest effectiveness in reversing immune features associated with progression towards the onset of labor.

Applying a multiplex mass cytometry approach to screen candidate drugs for PTB for their potential to reverse PTB-related immune signatures provides an efficient precision-medicine tool and can bridge the gap between in silico drug evaluation and clinical therapeutic intervention.

Aiswarya Sethumadhavan, PhD, Eric Meffre Lab

Altered autoreactive B cell selection during human pregnancy

Aiswarya Sethumadhavan, Nicolas L. Chamberlain, Sally Yraita, Stephanie Gaw and Eric Meffre

Fetal-maternal interface is a complex system regulated by many tolerance mechanisms. The fetal antigens released into the maternal immune system are processed by maternal antigen presenting cells and presented to the T cells to establish placental immune tolerance. Several studies have reported a critical role for B cells during pregnancy. Moreover, using transgenic mouse models, previous studies have shown that hormones, such as estrogen and prolactin may impact autoreactive B cell selection. Despite these observations, it remains unknown whether elevated concentrations of estrogen, progesterone, and prolactin during pregnancy interfere with autoreactive B cell counterselection in humans. To evaluate the impact of pregnancy on early B cell tolerance

checkpoints, we analyzed the reactivity of recombinant antibodies cloned from single transitional and mature naïve B cells isolated from women in their third trimester of pregnancy. Our preliminary data suggest that while the frequency of autoreactive mature naïve B cells remained low during pregnancy, central B cell tolerance was abrogated and resulted in the production of many autoreactive transitional B cells. We speculate that the broadening of the BCR repertoire during pregnancy will favor the presentation of fetal antigens by decidual B cells to T cells to ensure tolerance at the fetal-maternal interface.

Simone Thair, PhD, Purvesh Khatri Lab

Prospective multi-site validation of 11-gene host response signature for influenza diagnosis

Simone A. Thair, Maryam Shojaei, Tanya Novak, Lara Murphy Jones, Steven Schaffert, Damon Eisen, Win Sen Kuan, Rahul Santram, Mark Gillett, Kevin Lai, Anthony McLean, Sally Toh, Margaret Newhams, Adrienne Randolph**, Benjamin Tang**, Purvesh Khatri**

There is no single diagnostic that can identify influenza from other viral or bacterial respiratory infections and a biomarker to predict risk of severe outcomes is urgently needed. Objectives: To validate influenza diagnosis accuracy of our previously described host-immune peripheral blood-based 11-gene signature (Influenza Meta Signature (IMS)) in two prospective cohorts. To test if integrating detection of influenza viral RNA in blood with the IMS will be prognostic. Methods: 699 adults (652 suspected influenza, 47 healthy controls (HC)) across 10 Australian community or hospital clinics (AUS) and 182 critically ill pediatric patients across 28 US pediatric intensive care units with suspected influenza (PICFLU study) were enrolled. 11 IMS and 15 influenza genes were assayed using NanoString from peripheral blood. We defined the Blood Flu Score (BFS) from the 15 influenza genes. Results: AUS: The IMS score accurately distinguished nasopharyngeal swab RT-PCR for influenza patients from HC (AUROC=0.95, 95%CI:0.94−0.96), bacterial infections (AUROC=0.88, 95%CI:0.86−0.90), other viral infections (AUROC=0.77, 95%CI:0.74−0.79) and those with no pathogen detected (NPD)(AUROC=0.83, 95%CI:0.81−0.85). The IMS and BFS scores were positively correlated (r=0.47, p<2.2e-16). However, some patients had high BFS but not severe infection. The ratio of BFS-to-IMS was significantly higher in patients admitted to ICU compared to those who were not (p=7.1e-05). PICFLU: The BFS-to-IMS ratio's prognostic value replicated; it was significantly and increasingly higher in patients who progressed to ARDS (p=6.3e-03); MODS (p=4.5e-03); ECMO, (p=1.1e-04) and mortality (p=2.2e-04) versus those who did not. This validates the accuracy of IMS and potential prognostic value of the BFS-to-IMS ratio.

Kattria van der Ploeg, PhD, Pras Jagannathan Lab

Malaria-exposed Ugandans exhibit a differential SARS-CoV-2-specific T cell response

Kattria van der Ploeg, Karen B. Jacobson, John Rek, Felistas Nankya, Jessica Briggs, Saki Takahashi, Adam S. Kirosingh, Diego A. M. Mori, Kenneth Musinguzi, Isabel Rodriguez-Barraquer, Bryan Greenhouse, Upinder Singh, Moses R. Kamya, and Prasanna Jagannathan

While SARS-CoV-2 and its interaction with the immune response has been well-studied in resource-rich areas, there are still many unanswered questions about how it affects malaria-endemic areas in sub-Saharan Africa. Compared to other global regions, during the pandemic, hospitalization and death rates have been reported to be much lower in these areas. One possible explanation could be

differential immune responses due to different infectious exposures such as malaria. Using samples collected from SARS-CoV-2 exposed Ugandan adults, and similarly aged adults from California, we investigated the SARS-CoV-2-specific T cell response by intracellular cytokine staining (ICS) and an activation-induced marker (AIM) assay. Overall, we found that COVID-19 seropositive Ugandans have a diminished SARS-CoV-2-specific T cell response compared to convalescent Californians. IFNγ- and TNFα-producing T cells were predominant in the Californian cohort early on and months after initial infection. However, very low or no IFNγ and TNFα production was found in the Ugandan cohort. Furthermore, Ugandans have a heightened IL-10-producing CD4+ regulatory response after polyclonal stimulation. Ongoing research will investigate whether and how other pathogenic exposures, specifically malaria, may influence the T cell response. We hypothesize that previous exposure to malaria mitigates infection with SARS-CoV-2 due to a more 'tolerized' immune response. Potential explanations for our findings include epitope cross-reactivity or down-modulation of an inflammatory response that is implicated in severe COVID-19. Identifying differences in immune responses across populations will be important for future therapeutic innovations and vaccine development.

Fu-Chen Yang, PhD, Chaitan Khosla Lab

Identifying the pathogenically relevant location of active TG2 and gluten-presenting cells in celiac disease duodenal biopsies

Fu-Chen Yang, Harrison Anthony Besser, Chaitan Khosla

Celiac disease (CeD) is an autoimmune disorder that occurs in response to the ingestion of wheat and has no medical therapy aside from a gluten-free diet. In CeD, transglutaminase 2 (TG2) carries out the deamination of glutamine residues in gluten peptides. This chemical changes allows the gluten peptides to be presented by antigen-presenting cells on the disease-associated protein HLA-DQ2/8, bringing about T-cell activation and, ultimately, mucosal injury. Many biological functions have been attributed to TG2, but the pathogenically relevant location of active TG2 remains to be determined. Moreover, increased intestinal CD11c+ dendritic cells (DCs) capable of effectively activating gluten-specific T cells have been observed in inflamed duodenum from celiac patients. Here, our data addressed the location of active TG2 that renders gluten a toxic material in the small intestine of celiac patients. Moreover, we have identified that DC was the critical population in the uptake of TG2-gluten complex in the duodenum.

Hong Zheng, PhD, Purvesh Khatri Lab

Systems immunology profiling of treatment-naïve children with multisystem inflammatory syndrome

Hong Zheng, Lael Yonker, Michele Donado, Ben Solomon, Brittany Boribong, Yael Gernez, Bernard Kinane, Holden Maecker, Natalia Sigal, Ann Arvin, Katja Weinacht, Purvesh Khatri

Multisystem inflammatory syndrome in children (MIS-C) is a severe post-infectious complication occurring weeks after SARS-CoV-2 infection. The exact mechanisms leading to immune dysregulation and organ damage remain incompletely understood. Progress in understanding the immunopathology underlying MIS-C has been halted by limited availability of pre-treatment patient

samples and confounding effects of immunomodulatory treatment.

In this study, we restricted enrollment to treatment-naïve patients with MIS-C and used a systems biology approach combining CyTOF, single cell transcriptomics, and serum cytokine profiling to dissect how immune responses in children with MIS-C differ from children with mild SARS-CoV2 infection, adults with severe COVID-19 and healthy individuals. We also integrated single cell transcriptomics datasets from post-treatment MIS-C samples to study how immune responses change along disease course.

We identified increased inflammation and antigen presentation across multiple immune cell types in MIS-C patients. Importantly, in PBMCs of MIS-C patients, we identified a distinct subset of proinflammatory monocytes, with increased expression of interferon gamma response genes combined with a signature of enhanced complement expression, antigen processing and presentation, which was not observed in post-treatment MIS-C samples. Interestingly, this monocyte population bears resemblance to a subset of monocytes that emerges after the BNT162b2 mRNA vaccine booster. In addition, in PBMCs of MIS-C patients, we identify increased proportion of proliferating T/NK cells, suggesting distinct T cell expansions in MIS-C. T and NK cells in MIS-C samples also showed increased cell cytotoxicity markers.

Taken together, treatment-naïve MIS-C samples display distinct monocyte clusters, activated antigen presentation and complement expression, and increased T and NK cell cytotoxicity, which may account for the clinical presentation of MIS-C.

Yu Zhu, PhD, Eugene Butcher Lab

Venous reprogramming of tumor endothelium by COUP-TII expression stimulates anti-tumor immunity and enhances immunotherapy

Yu Zhu, Kevin Brulois, Theresa Dinh, Junliang Pan, Eugene Butcher

Scarcity of anti-tumor T cells in tumors poses a significant obstacle to cancer immunotherapy. To overcome this challenge, we focused on targeting the tumor vasculature, a critical gatekeeper of immune cell trafficking. We investigated a vessel-targeting approach that induces capillary-to-venule "reprogramming" to inhibit capillary tumor-promoting activities while enhancing T cell-recruiting functions. Ectopic expression of the nuclear receptor transcription factor COUP-TFII in tumor endothelial cells (ECs) led to endothelial acquisition of venular features and functions and a loss of blood capillaries. Lineage tracing confirmed that COUP-TFII-driven EC reprogramming occurred in a cell-autonomous manner. Capillary-to-venule reprogramming induced by COUP-TFII increased the recruitment of anti-tumor T cells into the tumor tissue, resulting in significantly reduced tumor burden in mouse breast and pancreatic tumor models. Moreover, COUP-TFII-driven EC reprogramming sensitized tumors to both immune checkpoint blockade and adoptive cell transfer therapy. Our findings suggest that reprogramming tumor vessels from capillaries into venules could be a promising strategy to enhance endogenous and therapeutic anti-tumor T cell responses.

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