Presenter: Abt, Michael

T regulatory cells support fecal microbiota transplantation for treatment of Clostridioides difficile by limiting type 1 inflammation

Michael Abt, Zahidul Alam, Jeffrey Maslanka, Joshua Denny, Kyle Bittinger

University of Pennsylvania

Fecal microbiota transplantation (FMT) is a successful therapeutic strategy for treating Clostridioides difficile infection. Despite remarkable efficacy, implementation of FMT therapy is limited and the mechanism of action remains poorly understood. Our previous work demonstrated a critical role for the immune system, specifically CD4+ Foxp3+ T-regulatory cells in enabling FMT engraftment and subsequent resolution of C. difficile infection. Here, we sought to elucidate the effector mechanism of action employed by T regulatory (Treg) cells to support FMT-mediated resolution of C. difficile infection. At the time of FMT, C. difficile infected mice have increased frequency and number of IL-10 expressing CD4+ Foxp3+ Treg cells in the colon compared to uninfected mice. Further, mice deficient in IL-10 signaling by germline deletion or antibody-mediated blockade fail to resolve C. difficile infection following FMT, phenocopying our observation in Treg cell depleted Foxp3-DTR mice. The depletion of Treg cells in C. difficile-infected Foxp3-DTR mice induces a robust type 1 immune response in the colon characterized by IFN-γ producing TH1 and type 1 innate lymphoid cells. Neutralization of IFN-γ following Treg cell depletion restores the capacity of FMT to resolve C. difficile infection. Combined these data support a mechanism by which IL-10 released by Treg cells limits IFN-γ driven intestinal inflammation thereby supporting an intestinal microenvironment receptive to FMT engraftment. These data demonstrate that the host's immune status can dictate the success of microbiota-based therapeutics to treat disease.

Presenter: Ahmed, Nasiha S.

Bromodomain protein 9 (BRD9) regulates interferon-stimulated genes during macrophage activation via cooperation with BET protein BRD4

Nasiha S. Ahmed, Jovylyn Gatchalian, Josephine Ho, Mannix J. Burns, Nasun Hah, Zong Wei, Michael Downes, Ronald M. Evans,
and Diana C. Hargreaves

Salk Institute for Biological Sciences

Macrophages induce a number of inflammatory response genes in response to stimulation with microbial ligands. In response to endotoxin Lipid A, a gene activation cascade of primary followed by secondary response genes is induced. Epigenetic state is an important regulator of the kinetics, specificity, and mechanism of gene activation of these two classes. In particular, SWI/SNF chromatin remodeling complexes are required for the induction of secondary response genes, but not primary response genes, which generally exhibit open chromatin. Here we show that a recently discovered variant of the SWI/SNF complex, the non-canonical BAF complex (ncBAF), regulates secondary response genes in the interferon (IFN) response pathway. Inhibition of bromodomain-containing protein 9 (BRD9), a subunit of the ncBAF complex, with BRD9 bromodomain inhibitors (BRD9i) or a degrader (dBRD9), led to reduction in a number of interferon-stimulated genes (ISGs) following stimulation with endotoxin lipid A. BRD9-dependent genes overlapped highly with a subset of genes differentially regulated by BET protein inhibition with JQ1 following endotoxin stimulation. We find that the BET protein BRD4 is co-bound with BRD9 in unstimulated macrophages and co-recruited upon stimulation to ISG promoters along with STAT1, STAT2, and IRF9, components of the ISGF3 complex activated downstream of IFNAR stimulation. In the presence of BRD9i or dBRD9, STAT1, STAT2, and IRF9 binding is reduced, in some cases with reduced binding of BRD4. These results demonstrate a specific role for BRD9 and the ncBAF complex in ISG activation and identify an activity for BRD9 inhibitors and degraders in dampening endotoxin- and IFN-dependent gene expression.

Presenter: Akhade, Ajay Suresh

A non-canonical role of caspase-1 in regulating bacterial physiology and antimicrobial resistance

Ajay Suresh Akhade, Gabriela Verdezoto Mosquera, Mario L. Arrieta-Ortiz, Amardeep Kaur, Eliza J.R. Peterson, Nitin S. Baliga,
Kelly T. Hughes and Naeha Subramanian
Institute for Systems Biology

Caspase-1 is a key effector molecule involved in inflammasome activation and has a well-established role in restricting the growth of intracellular pathogens like Salmonella by triggering a form of cell death called pyroptosis. Here we reveal a non-canonical, cell death independent role for caspase-1 in controlling the transcriptional state and drug resistance of intracellular Salmonella. Using Pathogen-sequencing, a method for sensitive transcriptional profiling of miniscule numbers of intracellular bacteria from infected macrophages, we show that that caspase-1 regulates key processes involved in survival and antimicrobial susceptibility of intracellular Salmonella. Host caspase-1 increased susceptibility of Salmonella to endogenous cationic antimicrobial peptides, as well as to a cationic polypeptide antibiotic used as a last-line drug in Gram-negative bacterial infections. These effects of caspase-1 were independent of its enzymatic activity but dependent on its ability to repress activation of a two-component signal transduction system in intracellular bacteria. These effects were also independent of caspase-11. Our data suggest a "backup" role for caspase-1 in dampening antimicrobial resistance of intracellular Salmonella, which evade initial innate immune detection and restriction by caspase-1. These findings also reveal the role of a key innate immune effector in altering pathogen physiology, broadening our view of host-pathogen crosstalk with possible implications for targeting caspase-1 in host-directed therapy to combat antimicrobial resistance.

Presenter: Apostol, April

Fetal HSCs respond to inflammation during maternal infection with Toxoplasmosis

April Apostol, Kelly Otsuka, Jasmine Posada, Diego Lopez, Kirk Jensen, Anna Beaudin

UC Merced

Infection in the adult organism drives cytokine-mediated inflammation that directly influences hematopoietic stem cell (HSC) function and differentiation within the bone marrow, but much less is known about the fetal hematopoietic response to maternal infection during pregnancy. Here, we investigated the fetal hematopoietic response to maternal Toxoplasma gondii (T. gondii) infection. T. gondii is an intracellular parasite that elicits Type II, IFNγ-mediated maternal immunity to prevent vertical transmission and promote parasite clearance. The production of excessive IFNγ during congenital toxoplasmosis has dire consequences for the developing fetus, such as lowered birth weights and premature abortion, but the effects to the developing immune system and the signals that mediate these interactions have not been investigated previously. Our examination illuminates that the fetal inflammatory repertoire is distinct from the maternal response and is directly influenced by parasite virulence. We show that maternal IFNγ crosses the fetal-maternal interface and is perceived directly by fetal HSCs, and the response of fetal HSCs is dependent on the fetal IFNγ receptor. Functionally, the heterogenous fetal HSC pool responds to aberrant inflammation with virulence-dependent changes in proliferation, long-term multi-lineage reconstitution, and self-renewal potential. By directly comparing the effect of maternal IFNγ injection with congenital T. gondii infection of varying virulence, our observations delineate both a direct effect of IFNγ on fetal HSCs and illuminate the independent role of additional inflammatory cytokines in driving the expansion of downstream hematopoietic progenitors. Finally, in direct contrast to the adult hematopoietic response to infection, exposure to inflammation in utero did not diminish fetal HSC function in response to mild infection. Our findings provide insight into the cues that direct fetal hematopoiesis in response to inflammation and begin to tease apart inflammatory cues that promote a beneficial hematopoietic response versus those that may ultimately be detrimental.

Presenter: Babirye, Janet Peace

Shigella flexneri activates CD4 and CD8 T cells which may confer resistance against secondary infection

Janet Peace Babirye, Justin L. Roncaioli, Katherine A. Deets, Dmitri I. Kotov, Russell E. Vance

University of California, Berkeley

Shigella species are the cause of bacillary dysentery and are responsible for significant global morbidity and mortality. There is currently no licensed vaccine against Shigella despite extensive efforts. A major barrier has been the lack of an animal model that can recapitulate clinical outcomes of infection. Recent work showed that mice deficient in the NAIP-NLRC4 inflammasome are susceptible to shigellosis and display similar clinical manifestations of disease observed in human infection. We hypothesized that T cells are activated during primary Shigella infection and may protect against secondary challenge with the same Shigella strain. We adoptively transferred OT-1 CD8 T cells into NAIP-NLRC4 deficient mice and infected them with Shigella flexneri strains expressing Ova and 2W one-day post transfer. We observed that the OT-1 CD8 T cells are activated and proliferate by 7 days post infection. We also observed that CD4 T cells from infected mice secrete interferon gamma upon in-vitro re-stimulation with 2W peptide. Our findings provide evidence for activation of CD4 and CD8 T cells upon Shigella infection and suggest a probable role of T cells in adaptive immune response to Shigella infection. This work may provide insight on adaptive immune responses against Shigella spp and enable successful vaccine design.

Presenter: Beppler, Casey

Altered synapse antigen scanning in CAR T cells

Casey Beppler, John Eichorst, Kyle Marchuk, En Cai, Carlos A. Castellanos, Venkataraman Sriram, Kole T. Roybal and Matthew F. Krummel

Department of Pathology and ImmunoX, UCSF; Biological Imaging Development CoLab, UCSF; Department of Microbiology and Immunology, UCSF;
Sandler Asthma Basic Research Center, UCSF; Foundery Innovations; Parker Institute for Cancer Immunotherapy; Chan Zuckerberg Biohub;
Helen Diller Comprehensive Cancer Center

T cells typically recognize their ligands using microvilli to scan for cognate antigen and scaffold T cell receptors (TCRs) during antigen recognition. Conventional chimeric antigen receptors (CARs) are often built using single-chain variable fragments (scFvs) with far greater affinity than that of natural TCRs. The implications of this for T cell function are not well understood. Using lattice light-sheet and synaptic contact mapping total internal reflection fluorescence microscopy, we studied the membrane dynamics in cells bearing anti-human epidermal growth factor receptor 2 (HER2) CARs and found these hyper-stabilized microvillar contacts relative to TCRs. While these CARs also impaired synapse resolution, a monomerized CAR with a lower affinity scFv rescued synapse dynamics and improved antigen-dose discrimination. The dimeric low-affinity CAR improved synapse dynamics and minimized early indicators of exhaustion, while maintaining or improving effector functions. This work highlights the need to consider designing CAR binding dynamics that more closely resemble natural TCR antigen sensing to optimize T cell quality and function.

Presenter: Billipp, Tyler

Tuft cell-derived acetylcholine regulates epithelial fluid secretion during homeostasis and Type 2 immunity

Tyler Billipp, Webeck Lily, Sargent Derek, Jakob von Moltke

University of Washington

The intestinal epithelium maintains a barrier against microbiota, pathogens, and environmental insults through the secretion of fluid and mucus. The Type 2 immune response to parasitic helminths or the metabolite succinate augments the epithelial barrier via goblet cell hyperplasia and increased mucus production. Tuft cells, rare chemosensory epithelial cells which sense helminths to initiate the Type 2 response, express the enzyme ChAT required to synthesize acetylcholine (ACh). ACh is a potent inducer of epithelial fluid and mucus secretion. We find that succinate induces rapid fluid and mucus secretion in the small intestine dependent on tuft cells, tuft-derived ACh, and independent of enteric neurons. Fluid secretion is enhanced during Type 2 inflammation, consistent with the observed increase in ChAT+ tuft cells. Upon infection with the hookworm Nippostrongylus brasiliensis, tuft-specific ChAT-deficient mice suffer delayed worm clearance despite an otherwise robust Type 2 response. Our findings suggest that upon sensing of luminal signals produced by helminths and microbes, tuft cells stimulate an epithelium-intrinsic effector unit composed of enterocytes and goblet cells to rapidly respond with fluid and mucus secretion. This response is amplified by the epithelial remodeling that occurs during the Type 2 response, contributing to anti-helminth immunity.

Presenter: Bod, Lloyd

Tumor-associated B cells: Friends or Foes?

Lloyd Bod, Lloyd Bod, Jingwen Shi, Elena Torlai Triglia, Alexandra Schnell, Yoon-Chul Kye, Johannes Fessler, Juhi R. Kuchroo,
Rocky M. Barilla, Sarah Zaghouani, Elena Christian, Toni Marie Delorey, Kanishka Mohib, Sheng Xiao, David M. Rothstein, Orit Rozenblatt-Rosen, Arlene H. Sharpe, Lionel Apetoh, Aviv Regev, Vijay K. Kuchroo

Harvard Medical School and Brigham and Women's Hospital

As one of the main populations of the immune system, B cells exert a key role in both the innate and adaptive branches of immunity. Depending on the context, different specialized B cell subsets are solicited and engage a various range of responses. Beyond their ability to mediate humoral response, B cells are potent antigen presenting cells and could provide co-stimulatory or co-inhibitory signals and secrete cytokines modulating the immune responses. In cancer, B cells represent the second most abundant tumor-infiltrating lymphocyte and therefore might play an important role in modulating the immune response to cancer. However, it remains ambiguous how B cells orient the anti-tumor immunity, indicated by divergent conclusions in studies addressing this question. A better understanding of the tumor-infiltrating B cell subsets remains essential to dissect how they influence cancer immunity. Using both bulk and single-cell RNA sequencing (scRNAseq) technologies combined with high-throughput flow cytometry, we investigated tumor-associated B cell diversity and potential role on tumor progression. Our goal is to better identify pro- and anti- tumor B cells to design therapies targeting one of these subsets and improve clinical outcome.

Presenter: Boothby, Ian

Early Life Inflammation Primes a Th2-Fibroblast Niche in Skin

Ian Boothby, Maxime J. Kinet, Devi P. Boda, Elaine Y. Kwan, Sean Clancy, Jarish N. Cohen, Ireneusz Habrylo, Margaret M. Lowe, Mariela Pauli,
Ashley E. Yates, Jamie D. Chan, Hobart W. Harris, Isaac M. Neuhaus, Timothy H. McCalmont, Ari B. Molofsky, and Michael D. Rosenblum
UCSF

Inflammation early in life can prime the local immune milieu of peripheral tissues, causing lasting changes in immunologic tone that confer disease protection or susceptibility. The cellular and molecular mechanisms that incite changes in immune tone in many nonlymphoid tissues remain largely unknown. We find that time-limited neonatal inflammation induced by transient reduction of neonatal regulatory T cells (Tregs) causes a dramatic dysregulation of subcutaneous tissue in murine skin, accompanied by the selective accumulation of Th2 cells within a distinct microanatomic niche. Th2 cells are maintained into adulthood through interactions with a fibroblast population in skin fascia that we refer to as Th2-interacting fascial fibroblasts (TIFFs), which expand in response to Th2 cytokines to form subcutaneous fibrous bands. Activation of the Th2-TIFF niche by neonatal inflammation primes skin for altered reparative responses to wounding. We further identify fibroblasts in healthy human skin expressing the TIFF transcriptional signature and find these cells at high levels in eosinophilic fasciitis, an orphan disease characterized by inflammation and fibrosis of the skin fascia. Taken together, these data define a novel Th2 niche in skin, functionally characterize a disease-associated fibroblast population, and suggest a mechanism of immunologic priming whereby inflammation early in life creates networks between adaptive immune cells and stromal cells, establishing an immunological set-point in tissues that is maintained throughout life.

Presenter: Bousbaine, Djenet

A conserved Bacteroidetes antigen induces anti-inflammatory intestinal T lymphocytes

Djenet Bousbaine, Laura I. Fisch†, Mariya London†, Preksha Bhagchandani, Mark Mimee, Scott Olesen, David VanInsberghe, Mathilde Poyet,
Ross W. Cheloha, John Sidney, Jingjing Ling, Aaron Gupta, Timothy K. Lu, Alessandro Sette, Eric J. Alm, James J. Moon, Daniel Mucida,
Angelina M. Bilate and Hidde L. Ploegh**

Stanford University

The microbiome contributes to the development and maturation of the immune system. In response to commensal bacteria, CD4+ T cells differentiate into functional subtypes with regulatory or effector functions. Intraepithelial lymphocytes in the small intestine that express CD4 and CD8αα homodimers (CD4IELs) show regulatory properties and promote tolerance against dietary antigens. Development of CD4IELs depends on the microbiota, but the identity of the microbial antigens recognized by CD4IELs remains unknown. We identified β-hexosaminidase, a conserved enzyme across commensals of the Bacteroidetes phylum, as a driver of CD4IEL differentiation. In a mouse model of colitis, β-hexosaminidase-specific T cells protected against intestinal inflammation. Thus, T cells of a single specificity can recognize a variety of abundant commensals and elicit a regulatory immune response at the intestinal mucosa.

Presenter: Boutet, Marie

Memory CD8+ T cells mediate early pathogen-specific protection via localized delivery of chemokines and IFN[g] to clusters of CCR2+Ly6C+ monocytes

Marie Boutet, Zachary Benet, Erik Guillen, Caroline Koch, Saidi M'Homa Soudja, Fabien Delahaye, David Fooksman and Grégoire Lauvau

Albert Einstein College of Medicine

Memory CD8+ T (CD8+ TM) cells are essential adaptive effectors of immune defenses because they are extremely efficient at quickly sensing and killing intracellular pathogens and tumors. Previously, using mice immunized and secondary challenged with Listeria monocytogenes (Lm) as a model of infection, we established that host protection requires TNFα and reactive oxygen species (ROS) derived from CCR2+Ly6C+inflammatory monocytes and neutrophils, which formed "effector clusters" with CD8+ TM cells in situ. Furthermore, we discovered that CD8+ TM cells underwent early reactivation and differentiation into robust IFNγ+ cytolytic effector cells whether cognate antigen (Ag) was present or not, in response to IL-18 and IL-15 cytokines. IFNγ from reactivated CD8+ TM cells directly signaled to CCR2+Ly6C+ monocytes, enhancing their ability to express effector cytokines (TNFα), chemokines (CXCL9) and microbicidal functions (ROS) for protection. However, sterilizing immunity also requires CD8+ TM cells to recognize cognate Ag but it is not clear how cognate Ag recognition precisely enables IFNγ-dependent pathogen clearance and rapid protection of vaccinated host before clonal expansion occurs. Our most recent work (Boutet et al., Science Advances 2021) in distinct models of immunization established that cognate Ag recognition by CD8+ TM cells on dendritic cells initiates their rapid and coordinated production of a burst of CCL3, CCL4 and XCL1 chemokines under the transcriptional control of IRF4. We hypothesized that Ag-dependent CD8+ TM cell-derived chemokine production is necessary for rapid microbial pathogen containment through recruitment and sustained clustering of CCR2+Ly6C+inflammatory monocytes with CD8+ TM cells and Ag-presenting cells, and to enable the delivery of IFNγ for effective myeloid cell microbicidal response. By intravital microscopy imaging and in vivo monoclonal antibody labelling, we revealed that CD8+ TM cells undergo Ag-dependent arrest in splenic red pulp clusters of CCR2+Ly6C+ monocytes where microbial pathogens localize, and to which they locally deliver IFNγ and chemokines. IFNγ promotes upregulation of XCR1 and CCR5 chemokine receptors on monocytes, making them responsive to chemokines that induced robust antimicrobial activities for protection. However, and in contrast to our original hypothesis,, we found that CCR2+Ly6C+ monocytes form clusters in the splenic red pulp independently from cognate Ag and CD8+ TM cells. We show that monocyte clustering requires Gai-mediated chemotaxis. Collectively, our data reveal that cognate Ag on DCs mediates CD8+ TM cell arrest in infection foci where blood-derived CCR2+Ly6C+ monocytes have accumulated independently from CD8+ TM cell-chemotactic cues. We show that CD8+ TM cells deliver IFNγ and a set of cognate Ag–triggered chemokines, CCL3, CCL4, and XCL1. Both signals are necessary to drive full activation of CCR2+Ly6C+ monocytes and their secretion of TNFαand CXCL9. IFNγ signals make CCR2+Ly6C+ monocytes responsive to the chemokine signals that drive their microbicidal functions. Thus, rapid, and effective protective CD8+ TM cell responses requires spatially and temporally coordinated events that quickly restrict microbial pathogen growth through the local delivery of activating chemokines and IFNγ to CCR2+Ly6C+ clustered monocytes. Furthermore, we observed that monocyte clustering depends on multiple factors including bacterial cytosolic signals, dendritic cells and chemotaxis.

Presenter: Brown, Matthew

Primatized immune system mice created via robust engraftment of fetal rhesus macaque hematopoietic tissues in immune-deficient mice.

Matthew Brown, Christopher J Little; W. John Haynes; Liupei Huang; Cross Daffada; Bryce B Wolfe; Elizabeth Perrin; John A Simpson;
Jenna A Kropp Schmidt; Hayly M Hinkle; Logan T Keding; Dixon B Kaufman; James A Thomson; Thaddeus G Golos; Matthew E Brown

University of Wisconsin-Madison

Non-human primates (NHPs) represent one of the most important models for pre-clinical biomedical studies. In contrast with small animal models, however, widespread utilization of NHPs is restricted by cost, logistics, and availability. Therefore, we sought to develop a translational primatized immune system mouse model, akin to a humanized mouse, to allow for high-throughput in vivo experimentation leveraged to inform large animal immunology-based studies. We found that adult rhesus macaque mobilized blood (AMb) CD34+ enriched hematopoietic stem and progenitor cells (HSPCs) engrafted at low but persistent levels in immune-deficient mice harboring transgenes for human (NHP cross-reactive) GM-CSF and IL3, but did not in mice with wild-type murine cytokines lacking NHP cross-reactivity. To enhance engraftment, fetal liver-derived HSPCs were selected as the infusion product based on an increased CD34hi fraction compared to AMb and bone marrow. Coupled with co-transplantation of rhesus fetal thymic fragments beneath the mouse kidney capsule, fetal liver-derived HSPC infusion in cytokine-transgenic mice yielded robust multilineage lymphohematopoietic engraftment. The emergent immune system recapitulated that of the fetal monkey, with similar relative frequencies of lymphocyte, granulocyte, and monocyte subsets within the thymic, secondary lymphoid, and peripheral compartments. Importantly, while exhibiting a predominantly naïve phenotype, in vitro functional assays demonstrated robust cellular activation in response to non-specific and allogenic stimuli. This primatized mouse represents a viable and translatable model for the study of hematopoietic stem cell physiology, immune development, and functional immunology in NHPs.

Presenter: Bunda, Nicholas

Microbial experience influences anti-tumor immune responses

Nicholas Bunda, Marlee Busalacchi, Kristin Renkema

Grand Valley State University, Department of Biomedical Sciences

Cancer immunotherapy research is traditionally conducted with specific pathogen-free (SPF) mice, which mimic the immune system of a human newborn. This poses a challenge for research aimed at developing immunotherapies for adult immune systems. We utilized a mouse model that bridges this gap and may improve translatability of future immunotherapy research. By cohousing SPF mice with pet store mice, we generated a cohoused (CoH) mouse that more accurately reflects the microbial experience of an adult human immune system. We hypothesized that CoH mice would mount a more effective anti-tumor response when compared to SPF mice. We injected the mice with B16 melanoma to investigate CD8+ T cells in various tissues. Preliminary data suggests that CoH tumor weights are significantly reduced when compared to SPF mice on day 10 post-injection. Control of tumor growth may be related to differences in CD8+ T cell activation profiles. Additionally, interesting differences in PD-1 expression suggests that exhaustion markers may play a role in controlling tumor growth. Collectively, our results indicate that microbial experience influences anti-tumor immunity and that cohoused mouse models may prove to have valuable translational applications.

Presenter: Callaway, Perri

The V[g]9V[d]2 T cell response is modulated by inhibitory receptors with MHC-I ligands

Perri Callaway, Lila Farrington, Justine Levan, Felistas Nankya, Kate Naluwu, Kenneth Musinguzi, Emmanuel Arinaitwe, Moses Kamya, Grant Dorsey, Margaret Feeney

UCSF/UC Berkeley

Vγ9Vδ2 T cells (Vδ2s) are a subset of γδ T cells that are important in their response to both microbes and to malignant cells. In particular, Vδ2s are intrinsically reactive to the phosphoantigen HMBPP produced by P. falciparum, the causative agent of malaria. HMBPP is taken up by host cells and binds intracellularly to the molecules BTN2a1 and BTN3a1, which in turn causes conformational shifts that are recognized by the Vδ2 TCR, leading to Vδ2 degranulation and cytokine production. We have previously shown that chronic P. falciparum exposure leads to a decrease in the overall frequency of Vδ2s and TCR responsiveness but an increase in their expression of a number of different NK receptors, including CD16 and KIRs. Here we expand these studies to other NK receptors and show that blocking MHC-I, the ligand not only for KIRs but also LILRB1 and NKG2a, increased overall Vδ2 activation. We further show that LILRB1 expression on Vδ2s is elevated in individuals with chronic malaria exposure but that NKG2a is not. LILRB1 is also more likely to be expressed on CD16+ Vδ2s while NKG2a has a uniform distribution across Vδ2 subsets. Together, these results suggest that LILRB1 on Vδ2s is modulated by repeated P. falciparum exposure in a similar manner to CD16 and KIRs but that NKG2a is independent of these changes. These results also have implications for the field of immuno-oncology where Vδ2s are being investigated as cell therapies and monoclonal antibodies against KIRs, LILRB1 and NKG2a are being developed as checkpoint inhibitors.

Presenter: Cautivo, Kelly M.

Interferon gamma constrains type 2 lymphocyte niche boundaries during mixed inflammation

Kelly M. Cautivo, Peri R. Matatia, Carlos O. Lizama, Nicholas M. Mroz, Madelene W. Dahlgren, Xiaofei Yu, Julia Sbierski-Kind, Marcela T. Taruselli, Jeremy F. Brooks, Adam Wade-Vallance, Sofia E. Caryotakis, Anthony A. Chang, Hong-Erh Liang, Julie Zikherman, Richard M. Locksley, Ari B. Molofsky

University of California, San Francisco

Allergic immunity is orchestrated by group 2 innate lymphoid cells (ILC2s) and type 2 helper T (Th2) cells prominently arrayed at epithelial- and microbial-rich barriers. However, ILC2s and Th2 cells are also present in fibroblast-rich niches within the adventitial layer of larger blood vessels and similar boundary structures in sterile deep tissues, and it remains unclear if they undergo dynamic repositioning during immune perturbations. Here we used thick-section quantitative imaging to show that allergic inflammation drives invasion of lung and liver non-adventitial parenchymal regions by ILC2s and Th2 cells. During concurrent type 1 and type 2 inflammation, IFN-γ from broadly distributed type 1 lymphocytes directly blocked both ILC2 parenchymal trafficking and subsequent survival. ILC2 and Th2 cell confinement to adventitial niches limited mortality by the type 1 pathogen Listeria monocytogenes. Our results suggest that the topography of tissue lymphocyte subsets is coordinately regulated to promote appropriately timed and balanced immunity. Our ongoing work will investigate the mechanism through which IFN-γ impairs the trafficking and survival of ILC2s and Th2 cells, as well as define type 1 lymphocyte subsets, their topography and their functional impacts on parenchymal cells in mixed inflammation.

Presenter: Chang, Anthony

Defining a stromal niche for type 2-like lung regulatory T cells

Anthony Chang, Peri Matatia, Joanna Balcerek, Michael Rosenblum, Ari Molofsky

University of California, San Francisco

Regulatory T cells (Tregs) are traditionally thought of as critical negative regulators of systemic immune responses; however, their local roles in tissues such as the lung are being increasingly appreciated, where they can promote lung epithelial regeneration. Although tissue Tregs (tTregs) function are beginning to be understood, how lung tTregs are regulated, positioned, and maintained within their respective tissue niches remains unknown. Our group identified a stromal cell niche within the lung where adventitial fibroblasts (AFs), a mesenchymal stromal cell subset, regulate type 2 effector lymphocyte (e.g. ILC2s and Th2 cells), in part via the secretion of IL-33 and thymic stromal lymphopoietin (TSLP). Using 3D thick section imaging, we have shown that lung type 2-like tTregs (i.e. Gata3hi ST2+, KLRG1+) also localize to this niche, indicating AFs may regulate lung tTregs and their subsets. When co-cultured with lung AFs, lymphoid Tregs significantly increased proliferation, survival, and expression of type 2-like tTreg markers ST2 and KLRG1 in a contact-dependent manner. Additionally, AFs preferentially support ST2hi Tregs over ST2lo Tregs, as evidenced by higher proliferation, survivability, and ST2 and KLRG1 expression. Using CellphoneDB V2.0, I identified extracellular matrix (ECM)–integrin ligand–receptor pairings, such as ICAM, VCAM, and CD49d, that may mediate interactions between AFs and Tregs. Upon blocking all three in a co-culture system, I found a significant decrease in Treg proliferation and ST2 and KLRG1 expression. We hypothesize that lung AFs regulate the maintenance and differentiation of lung tTregs subsets, preferentially supporting type 2-like lung Tregs. Further work will begin to use genetic mouse models to specifically delete AFs or AF-derived signals to fully dissect the role lung AFs play in under both naïve and inflammatory settings. Together, our work aims to define a novel stromal niche within the lung that plays critical roles in the regulation and maintenance of lung type-2 Tregs.

Presenter: Chang, Evelyn

CD4 T-Cell Exhaustion Develops During Chronic Mycobacterium tuberculosis Infection

Evelyn Chang, Tomoyo Shinkawa, Samuel Behar

University of Massachusetts Chan Medical School, Department of Microbiology and Physiological Systems

Tuberculosis (TB) is the leading cause of death by a single infectious agent, surpassed in 2020 - 2021 only by SARS-CoV2. Following infection by Mycobacterium tuberculosis (Mtb), 5-10% of individuals will go on to develop active and contagious disease. Risk factors, such as HIV, malnutrition, and diabetes, increase the probability. However, for most cases, the mechanisms underlying this progression are poorly understood. CD4 T-cells are required for a protective immune response, and given their importance, we hypothesize that CD4 T-cell exhaustion develops late during infection, and the loss of T-cell function contributes to the progression from latent to active TB. The field of T-cell exhaustion has primarily defined CD8 T-cells in the context of cancer and chronic viral infections, so TB provides a unique opportunity to define CD4 T-cell exhaustion in the context of chronic bacterial infection. We use the murine model of TB to study CD4 T-cell function, and we find, late during infection, CD4s exhibit features of exhausted CD8s, such as increased inhibitory receptor (IR) expression and loss of cytokine production. Specifically, progenitor exhausted T-cells express both TCF-1 and PD-1, whereas terminally exhausted T-cells express PD-1 and TIM-3. Furthermore, we observe a loss of T-cell polyfunctionality that correlates with the increased IR expression. We find traditional methods of examining T-cell function and cytokine production are insufficient in enumerating the total population of antigen-specific T-cells, so we developed an adoptive transfer model, using TB antigen-specific (C7) T-cells, to study CD4 T-cell exhaustion during TB. These C7 T-cells display a similar phenotype to those of intact mice. Interestingly, transcriptional profiling of the exhausted T-cell populations shows the terminally exhausted, PD-1+ TIM-3+ T-cells express the most IFNγ. Using in vitro assays, we show that PD-1+ TIM-3- and PD-1+ TIM-3+ T-cells differ in their ability to restrict intracellular Mtb growth. Finally, we find that in the context of chronic TB, TCR affinity contributes to the development of exhaustion. These studies show that CD4 T-cell exhaustion should be considered as a factor that leads to the development of active TB.

Presenter: Chen, Dan

A critical partnership between microglia and CD4+ T cells promotes anti-tumor immunity to glioblastoma

Dan Chen

Salk Institute

Glioblastoma multiforme (GBM) is the deadliest form of brain cancer, for which there is currently no effective treatment. Despite success of immunotherapies such as checkpoint inhibitors (CPI) in treating some cancers, less than 8% of GBM patients responded to anti-PD-1 therapy. Thus, we need to understand what mechanisms underlie tumor resistance to immunotherapies. In our GBM mouse model, we have found that anti-CTLA-4 treatment greatly improved survival and suppressed tumor growth, but surprisingly, its efficacy was lost in mice that were depleted of CD4+ but not CD8+ T cells, suggesting that CD4+ T cells are critical for protection against GBM. This matches clinical data showing decreased populations of CD4+ T cells in patients with adverse clinical outcomes such as high-grade GBM. Our preliminary results from flow cytometry and single-cell RNA sequencing (sc-RNAseq) also suggested enhanced cytokine production (IFN?, TNFa, IL-2) in CD4+ T cells after anti-CTLA-4 treatment. Similarly, we found that microglia, the local sentinels of the central nervous system, also play a significant role in tumor control, as indicated by faster GBM growth and reduced number of tumor-infiltrating CD4+ T cells when mice are depleted of microglia by a CSF1R inhibitor (PLX3397). Additionally, microglia in GBM may serve as potential antigen-presenting cells, as shown by their high MHC class II (MHC-II) expression. Therefore, we postulate that microglia are necessary to sustain anti-tumor immunity by CD4+ T cells. Importantly, through a more detailed exploration of how CD4+ T cells and microglia interact to impact tumor control, our study will provide new insights for developing therapeutic strategies against “difficult-to-treat” cancers such as GBM.

Presenter: Chen, Irene P.

Viral E Protein Neutralizes BET Protein-Mediated Post-Entry Antagonism of SARS-CoV-2

Irene P. Chen, James E. Longbotham, Sarah McMahon, Rahul K. Suryawanshi, Jared Carlson-Stevermer, Meghna Gupta, Meng Yao Zhang,
Frank W. Soveg, Jennifer M. Hayashi, Taha Y. Taha, Victor L. Lam, Yang Li, Zanlin Yu, Erron W. Titus, Amy Diallo, Jennifer Oki,
Kevin Holden,
QCRG Structural Biology Consortium, Nevan Krogan, Danica Galonic Fujimori, Melanie Ott
Gladstone Institutes

Inhibitors of Bromodomain and Extra-terminal domain (BET) proteins are possible anti- SARS-CoV-2 prophylactics as they downregulate angiotensin-converting enzyme 2 (ACE2). Here, we show that BET proteins should not be inactivated therapeutically as they are critical antiviral factors at the post-entry level. Knockouts of BRD3 or BRD4 in cells overexpressing ACE2 exacerbate SARS-CoV-2 infection; the same is observed when cells with endogenous ACE2 expression are treated with BET inhibitors during infection, and not before. Viral replication and mortality are also enhanced in BET inhibitor-treated mice overexpressing ACE2. BET inactivation suppresses interferon production induced by SARS-CoV-2, a process phenocopied by the envelope (E) protein previously identified as a possible "histone mimetic." E protein, in an acetylated form, directly binds the second bromodomain of BRD4. Our data support a model where SARS-CoV- 2 E protein evolved to antagonize interferon responses via BET protein inhibition; this neutralization should not be further enhanced with BET inhibitor treatment.

Presenter: Courau, Tristan

Immune aging as a critical factor of tumor development and control

Tristan Courau, Molly Bassette, Nayvin Chew, Gabriella Reeder, Kelly Kersten, Bushra Samad, Leonard Lupin-Jimenez, Matthew Krummel

University of California, San Francisco - Department of Pathology

Advanced age is the most important risk factor for cancer incidence worldwide. While the role of the aging immune system in this increased incidence is not well understood, a growing body of experimental evidence has revealed widespread upregulation of inflammatory signals in mouse tissues with increasing age. Together, this suggests an important role for advancing age in the functionality of cancer immune surveillance. To understand the particularities of immune system aging in the context of cancer development and progression, we have performed CyTOF and single cell RNA sequencing to profile the tumor microenvironment (TME) and lymphoid organs of young and aged mice challenged with a variety of human-relevant tumor models. We observed that aging strongly impacts the composition of the host systemic immune system in mice, and preliminary data suggests an age-related role in immune surveillance of early tumor development. Indeed, when challenged with a small tumor burden akin to initial cancer development, young mice reproducibly reject tumors, whereas aged mice do not. Additionally, aging in the immune compartment of the TME seems to primarily influence the phenotype of Tumor-Associated Macrophages (TAMS), the most prominent immune population typically found in the mouse TME. Mechanistic investigation of aged TAMs suggests important differences in their phenotype and function, pointing to TAMs as key players in immune system aging and cancer surveillance. Together, these data suggest a critical role for an aging immune system in cancer control and progression, and highlight potential therapeutic targets for an aging immune system in the context of cancer.

Presenter: Couturier, Nicole

Surveying the dynamic phenotype of tumor infiltrating immune cells in a murine breast cancer model

Nicole Couturier, Marie Boutet, Wenjun Guo, Gregoire Lauvau

Albert Einstein College of Medicine

Breast cancer (BC) is the second leading cause of death in women of all ages each year with more than half a million deaths worldwide. Given its widespread impact, it is vital that new BC treatments and therapies continue to be explored. In our lab we use a BC model (referred to as MP5) that consists of mammary stem cells grown in 3D culture that we orthotopically inject into the mammary fat pad of a female mouse. In these cells, ex vivo CRISPR-Cas9 gene editing was used to introduce three clinically relevant mutations: loss-of-function of the tumor suppressor protein p53 (p53-/-), constitutive activation of PI3 kinase (PIK3CA), and loss of function of the tumor suppressor gene MLL3 (Mll3-/-), a methyltransferase. The combination of these three oncogenic mutations appears in 12% of human breast cancers, recapitulating the aggressive tumor growth and poor overall prognosis seen in patients. In mice, the cells grow into palpable tumors approximately 30 days after injection. Currently, this preclinical model is not yet fully described; therefore, we developed a 31-color high dimensional spectral flow cytometry panel to characterize the phenotype of tumor infiltrating leucocytes in the MP5 tumors. We monitored the kinetics of tumor infiltrating immune cells and their phenotypes by examining activation and fate mapping markers, as well as the expression of inhibitory and chemotactic receptors. Our results show that T cells quickly infiltrate early-stage tumors right after onset (~2 mm3) while macrophages represent the largest population at later stages of tumor development, between 100 mm3 and 1,000 mm3. In the lymphocyte population, we observed that CD4+ T cells and CD8+ T cells are present in equal proportions at all stages of tumor growth. Further analysis of the CD4+ T cells showed that there is a greater number and proportion of conventional CD4+ T cells (Tconv) than Foxp3+ regulatory CD4+ T cells (Treg). Interestingly, however, the effector marker KLRG1 and the inhibitory markers PD-1 and TIGIT are more highly expressed on Treg cells than on Tconv cells, especially in larger tumors, suggesting that Treg cells may contribute to immunosuppression. Additionally, we found that the F4/80+ macrophages are composed of two distinct subsets that either express or do not express the CD169 sialoadhesin. This latter subset of macrophages also expresses the inhibitory markers PD-L1 and PD-L2 which become more highly expressed as the tumor progresses in size, suggesting that these macrophages may be involved in promoting tumor growth. Altogether, these results are consistent with a model in which Treg cells infiltrate the tumor early on to establish tumor immunosuppression. We further speculate that during later tumor development, this promotes the emergence of an inhibitory macrophage population that quickly expands and accelerates tumor growth by maintaining an immunosuppressive environment.

Presenter: Curran, Riley

Ligand specificity and Intratumoral Program of Self-Specific Memory-Phenotype CD8+ T cells

Riley Curran, Christine Miller, Alok Joglekar, Peter Savage

University of Chicago

In mice, there exists a population of CD8+ T cells named “memory phenotype” (CD8-MP) cells, which comprise approximately 10% of the CD8+ T cell population. CD8-MP cells, like “true memory” (CD8-TM) cells primed by exposure to foreign peptides, are characterized by elevated surface expression of CD44, CD122 and an array of additional memory T cell markers. Unlike CD8-TM cells, which arise after exposure to pathogen or an inflammatory insult, CD8-MP cells exist in both specific pathogen free and germ-free mice with no history of pathogen exposure or manipulation. Recent work from our group suggests that many CD8-MP T cell clones exhibit overt reactivity to MHC-I-restricted self-ligands and initiate differentiation into the CD8-MP phenotype during maturation in the thymus, prior to their emigration to the periphery. We also demonstrated that distinct CD8-MP clones are recurrently enriched in oncogene-driven mouse prostate tumors, suggesting a potential role for self-specific CD8-MP cells in anti-tumor immunity. These advances highlight two major gaps in knowledge that have restricted progress in the field: the lack of knowledge regarding the identity of natural self-ligands recognized by CD8-MP cells, and the lack of faithful markers to directly identify self-specific CD8-MP cells in inflammatory settings such as tumors. Here, we aim to bridge these two gaps in knowledge utilizing novel approaches. To identify specific CD8-MP self-ligands, we and our collaborators are utilizing a high throughput antigen discovery platform, SABR (Signaling and Antigen-presenting Bifunctional Receptors), to screen libraries of MHC-I-restricted self-peptides using canonical CD8-MP-biased T cell clones. To identify unique markers expressed by CD8-MP cells within tumors, we are utilizing long-term CD8-MP transfer experiments and comparative RNA sequencing to identify distinct transcriptional programs that distinguish intratumoral CD8-MP cells from bona fide tumor-specific CD8+ T cells. Together, achieving these objectives will enable new research to examine the role of self-ligand recognition in CD8-MP differentiation, and will permit the direct enumeration and characterization of self-specific CD8-MP cells in murine and human cancers.

Presenter: Daly, Allison

Mechanistically Understanding the NF[k]B Immune Response to Bacterial Infection

Allison Daly, Ann-Jay Tong, George Yeh, Xin Liu, Stephen Smale

UCLA

My research aims to uncover molecular mechanisms that govern immune cell activation. The NFκB family of transcription factors [TFs] is responsible for the expression of hundreds of genes in response to lipopolysaccharides. Although much is known about NFκB, a major paradox in the field is that TFs from the same family, which recognize and bind to similar DNA motifs in vitro, are often expressed in the same cell yet bind to and activate different genes in vivo. This observation implies that novel mechanisms govern TF specificity. I aim to uncover the mechanisms individual subunits of the NFκB transcription family use to regulate subsets of genes. I am using genome wide analysis to begin addressing this question. With RNA-Seq, I look for genes that are dependent upon a specific subunit of NFκB. With ChIP-Seq, I can correlate the binding of TFs with expression dependency, investigate motif differences in preferential binding sites and understand the combinatorial binding of the NFκB subunits at the same DNA locus. So far, I have identified unique roles for individual NFκB subunits and am beginning to understand the mechanisms behind their gene regulation. Specifically, my lab has found p50, a subunit of the NFkB family, is responsible for the expression of a small set of genes involved in Th17- cell differentiation. I am currently using sequential ChIP-seq, EMSA and CRISPR to elucidate the molecular mechanisms involved in p50-dependent gene regulation.

Presenter: Davis, Caitlin

You Really Got a Hold on Me – the Consequences of High Affinity Binding for Developing Autoreactive T Cells

Caitlin Davis, Jacob Du, Alexander Berg, Alicia Freedman, Tyler Marie Deveau, Stefan Abreo, Gagandeep Chouhan, An Nguyen,
Andrea Leung, Imene Smati, Harkiran Bhasin, Leda Rasooli, Sarina Duong, Martha C. Zúñiga
UCSC

T-cell antigen receptors are generated by more or less random gene rearrangement events. Thus, some antigen receptors may be autoreactive. To avoid autoimmunity, developing thymocytes must undergo stringent selection events. During positive selection T cells that bind to self-MHC + antigenic peptide receive survival signals. Next, negative selection eliminates those thymocytes whose receptors bind to self-MHC + self-peptide too avidly. Positive and negative selection events yield CD4+ and CD8+ T cells that are MHC-restricted and tolerant to self-protein antigens. There is, however, a third possible fate for αβTCR+ thymocytes. Some thymocytes whose receptors bind with very high affinity to self-peptide/MHC complexes are not deleted but instead undergo "deviated differentiation" into intraepithelial (IEL) precursors. In some transgenic mouse models used to study negative selection, the negatively selecting ligand is expressed throughout the thymus, thereby leading to deletion of thymocytes prematurely at the DP stage, possibly before or simultaneously with positive selection. These models do not allow examination of selective events that occur after positive selection. A T-cell clone named BM3.3 was previously isolated from CBA (H-2k) mice immunized with H-2b cells. The TCR from this clone binds with high affinity to the H-2Kb MHC I molecule. While CD8 expression is required for positive selection of BM3.3 TCR-bearing thymocytes, it is not required for negative selection. We have examined the development of thymocytes in three types of transgenic mice: mice expressing the BM3.3 TCR alone, mice expressing the BM3.3 TCR and the H-2Kb class I MHC molecule in the thymic cortex (KQxBM3 mice), and BM3.3 TCR transgenic mice expressing H-2Kb in the medulla (KALxBM3 mice). There are equivalent percentages of DP thymocytes in BM3, KQxBM3, and KALxBM3 mice. This enables us to examine the fates of thymocytes that encounter their high-affinity ligand after positive selection. We have found that in KQxBM3 mice, CD8+ thymocytes develop normally. Indeed, KQxBM3 mice have more CD8+ thymocytes than do BM3 mice. By contrast, KALxBM3 mice have few to no mature CD8+ thymocytes. In contrast to BM3 and KQxBM3 mice, KALxBM3 thymii have an exaggerated number of CD4-CD8- (DN) thymocytes. Using CD5 as a marker of encounter with antigen we investigated the DN thymocytes. BM3 and KQxBM3 DN thymocytes are CD5lo/egCD62Lhi. BY comparison, the KALxBM3 DN thymocytes consist of two distinct populations: CD5lo/egCD62Lhi DNs and CD5int/hiCD62Llo (antigen experienced) DNs. The levels of CD62L on the CD5int/hiCD62Llo DN thymocytes are equivalent to the levels observed on DP thymocytes. The most parsimonious conclusion from these data is that the CD5int/hiCD62Llo thymocytes in KALxBM3 mice are derived from DP thymocytes during the course of negative selection. Thymocytes and T cells from BM3 transgenic mice can express two kinds of TCR on their surfaces – a TCR composed of the transgenic a and b chains and a TCR composed of an endogenous a chain paired with the transgenic b chain. To ensure that the phenotypes we observe are due exclusively to the expression of the transgenic BM3 TCR, we created rag-/- KALxBM3 (called KBBR) and KQxBM3 (called KQBR) mice and compared their thymocytes to those of rag-/- BM3 mice. The KBBR mice had elevated numbers of DN thymocytes and a significant fraction of these were CD5int/hiCD62Llo, just as is seen in KALxBM3 mice. Lymph nodes of KQxBM3 mice have CD4+ and CD8+ T cells. In contrast, KALxBM3 lymph nodes have CD4+ and DN T cells. The majority of these KALxBM3 CD4+ and DN T cells have a memory phenotype. ~40% of the DN T cells are PD-1+ and thus may be immunosuppressive. Finally, KALxBM3 and KBBR intestines also have higher numbers of CD8αα and DN IELs than do BM3 rag-/- and KQBR mice. A significant fraction of both CD8 aa and DN IELs in all four strains examined (BM3rag-/-, KQBR, KALxBM3, and KBBR) express PD-1. Collectively our data show that highly autoreactive thymocytes have one of three fates: deletion, becoming DN T cells that migrate to peripheral lymph nodes, and IELs that populate intestinal tissue.

Presenter: DeRogatis, Andrea

Evaluation of the trade-off between feather molt and innate immunity in the domestic chicken (Gallus gallus domesticus)

Andrea DeRogatis, Kirk Klasing

University of California, Davis

There are a variety of nutritionally costly life stages that must be balanced with the costs of the immune system. The process of feather growth in birds is characterized by a range of physiological shifts and is known to be expensive in terms of the nutrients required to produce quality feathers quickly. However, little is understood about the relationship between the costly processes of molt and immunity. We investigated both the systemic effects of molt on immunity as well as the impact of molt on innate immunity in order to clarify how the nutritional requirements of molt may alter investment in immunity. To evaluate systemic effects, chickens (Gallus gallus domesticus) were induced to molt using a combination of oral thyroxine (T4), a reduced calorie diet and a shift from a long day to a short-day schedule. Over the course of a six-week period, liver, spleen, and thymus samples were collected weekly from molting and control birds. Molting birds had significantly larger spleens and thymuses ($P < 0.05$) and throughout the course of molt both pro and anti-inflammatory cytokines were higher in molting birds ($P < 0.05$). Additionally, molting birds had decreased levels of splenic CD4+ T-cells compared to control birds. In the second experiment, innate immunity during molt was evaluated by administering an injection of lipopolysaccharide (LPS) three weeks after the onset of molt to initiate an acute phase response. Four hours after the LPS injection, tissue samples were collected for evaluation of cytokine expression using qPCR. Molt led to a significant increase in the size of both the spleen ($p < 0.05$) and the thymus ($p < 0.05$). Based on inflammatory cytokine expression levels, both molting and non-molting birds mounted a robust innate immune response demonstrating that innate immunity likely has higher priority over feather growth for nutritional resources. Our data supports that molting birds undergo broad physiological changes associated with immunity that are likely important for maintaining health during the complex process of molt. This research highlights how nutritional resources are preferentially used by the immune system over other physiological processes during molt. Understanding how nutrients are allocated to different physiological processes is an important factor to consider when thinking about the immune system for any species, not just chickens.

Presenter: DeRogatis, Julia

Targeting the PSGL-1 Immune Checkpoint Promotes Immunity to PD-1 Resistant Melanoma

Julia DeRogatis, Karla Viramontes, Emily Neubert, Monique Henriquez, Christian Guerrero-Juarez, Roberto Tinoco

University of California, Irvine

Immune checkpoint inhibitors have had impressive efficacy in some cancer patients, reinvigorating long-term durable immune responses against tumors. Despite the clinical success of these therapies, most cancer patients continue to be unresponsive, highlighting the need for novel therapeutic options. PSGL-1 has been shown to act as an immune checkpoint, inhibiting immune responses in a variety of disease models. However, previous work has yet to address whether PSGL-1 can be targeted therapeutically to promote tumor control. Using an aggressive melanoma tumor model, we targeted PSGL-1 in tumor-bearing mice and found increased effector CD4+ and CD8+ T cell responses and decreased Tregulatory cells in tumors. T cells exhibited increased effector functions, activation, and proliferation, which slowed tumor growth in mice after anti-PSGL-1 treatment. Blocking PD-1 in PSGL-1-deficient tumor-bearing mice led to an increased frequency of mice with complete tumor eradication. Our findings show that therapeutically targeting the PSGL-1 immune checkpoint can reinvigorate anti-tumor immunity and indicate that blocking PSGL-1 may represent a new therapeutic strategy for cancer patients.

Presenter: Diaz-Ochoa, Vladimir

NRAMP1 Contributes to Neutrophil-Mediated Killing of Intracellular Pathogens

Vladimir Diaz-Ochoa, Kristen L. Lokken, Lizbeth Camacho, Ariel Munoz, and Renée M. Tsolis
University of California Davis

The Natural Resistance Associated Macrophage Protein 1 (NRAMP1) is a prominent metal transporter in host defenses against pathogens. Prevailing scholarship holds that macrophages facilitate control of intracellular pathogens via NRAMP1 mediated metal starvation in the phagosome. We found that NRAMP1 in neutrophils also contributes to host control of bacterial pathogens. While investigating how vitamin A deficiency impairs immunity against systemic salmonellosis, we discovered that NRAMP1-deficient animals were equally susceptible to disseminated Salmonella as vitamin A-deficient, NRAMP1-proficient, mice. This data suggested that NRAMP1 mediated immunity to disseminated salmonellosis was dependent on vitamin A. Indeed, Vitamin A deficiency impaired infection-induced granulopoiesis, resulting in reduced expression of specific- and gelatinase granule components in neutrophils, including NRAMP1. Adoptive transfer of neutrophils from NRAMP1-proficient donors, but not NRAMP1-deficient donors, reduced the systemic Salmonella burden in vitamin A-deficient, NRAMP1-proficient, mice and in NRAMP1-deficient animals. Additionally, NRAMP1-deficient neutrophils displayed diminished killing of Salmonella ex vivo compared to NRAMP1-proficient neutrophils. Induction of NRAMP1 in neutrophils occurs at the terminal stages of neutrophil differentiation in the bone marrow. We generated a mouse that had NRAMP1 functionality in macrophages but lacked Nramp1 expression in neutrophils because of a genetic defect in neutrophil terminal differentiation (Cebpe-/- Nramp1+/+). We confirmed the NRAMP1 cellular phenotype by infecting phagocytes isolated from this new mouse strain. Salmonella growth was under the control of NRAMP1 in peritoneal macrophages but not dependent on CEBPe whereas bone marrow neutrophils where equally susceptible to a higher intracellular Salmonella burden without NRAMP1 or CEBPe. In vivo, we found that susceptibility to a disseminated Salmonella infection was high in the Cebpe-/- Nramp1+/+ animals compared to wild type controls; a difference that was largely ablated in the whole body NRAMP1 deficient mouse in which all animals exhibited a much higher systemic burden of Salmonella. During infection with another vacuolar intracellular pathogen, Brucella abortus, we found that NRAMP1 contributed to a reduction in Brucella burden of the placenta, in which neutrophils also play a prominent role. Collectively, these data indicate that NRAMP1-deficiency impairs control of intracellular pathogens by blunting neutrophil-mediated host defenses.

Presenter: Dou, Diana R.

Xist complex drives sex-biased autoimmunity

Diana R. Dou, Yang Zhao, Jin Xu, Yanyan Qi, Sarah Chang, Allan Feng, Anton Wutz, PJ Utz, Howard Y. Chang

Center for Personal Dynamic Regulomes and Program in Epithelial Biology, Stanford University School of Medicine, Dermatology, Stanford, CA 94305

Autoimmune diseases disproportionately impact females 4x more than males, but the underlying basis for this sex-biased prevalence remain poorly understood. Existing studies implicating hormonal differences fail to reconcile the increased susceptibility to autoimmune diseases in Kleinfelter males, who have two X chromosomes (XXY) like females (XX). Since the long noncoding RNA (lncRNA), Xist, is expressed only in females for X chromosome silencing and the Xist ribonucleoprotein particle (RNP) contains many autoantigens associated with autoimmune diseases, we hypothesize that Xist RNPs stimulate a proinflammatory environment leading to increased autoimmunity. To study the influence of Xist RNPs on immune responses, we used a unique tgXist-mouse to confer inducible tgXist expression in male animals in the pristane-induced systemic lupus erythematous (SLE) model. Our studies suggest that: 1. Pristane-treated tgXist/Xist mice display heightened production of SLE-associated antibodies compared to wild-type male mice, 2. tgXist expression in male animals promotes a more female-like epigenetic and expression profile in CD4+ T-cells, and 3. tgXist-expressing male mice develop phenotypic disease with organ involvement more similar to control female than control male mice. Altogether, the data implicate a novel role for the autoantigen-dense Xist RNPs as immune complexes that may activate immune signaling cascades leading to autoimmunity.

Presenter: Ewing-Crystal, Nathan A.

CNS Fibroblasts Define Long Lived Cortical Immune Niches in Mouse Models of Stroke

Nathan A. Ewing-Crystal, Nicholas M. Mroz, Thomas D. Arnold, Ari B. Molofsky

University of California, San Francisco

Ischemic stroke is a leading cause of death worldwide with no approved treatments post-injury. This therapeutic gap reflects a lack of understanding of cell- and tissue-level interactions that drive and/or impair long-term recovery. Immune cells infiltrate ischemic lesions, but their roles and the signals that maintain them in the central nervous system (CNS) remain largely unknown. Similarly, long term CNS remodeling after stroke involves reactive astrocytic gliosis. However, recent evidence suggests additional stromal cells also respond and accumulate with CNS damage, though the ontogeny and function of this stromal response is not understood. Given known roles for stromal cells in maintaining and shaping immune responses outside of the CNS, we are investigating this stromal response in ischemic stroke through the lens of stromal-immune interactions. Using either a photothrombotic injury or a transient middle cerebral artery occlusion model, we have found that ischemia induces the proliferation and expansion of stromal cells that are derived from collagen-expressing fibroblasts and lack pericyte markers (e.g. desmin) but express markers of activated fibroblasts. While fibroblasts are restricted to perivascular niches and meningeal borders in the resting CNS, lesional fibroblasts surround the ischemic region by 14 days post injury (dpi) and subsequently persist for months. Additionally, accumulating lymphocytes closely and specifically associate with these fibroblasts, raising the possibility that stromal niches support lymphocyte survival and/or expansion. In future work, we will examine the result of genetically perturbing these stromal niches by specifically deleting lesional fibroblasts and modulating fibroblast-lymphocyte communication pathways, using immunophenotyping as a proximal output and behavior as a terminal output.

Presenter: Fesneau, Olivier

Identification of a subset of tumor-reactive CD8 TILs expressing NKG2A in HNSCC and CRC.

Olivier Fesneau, Kimberly Samson, Venkatesh Rajamanickam, David Ross, Thomas Duhen

Earle A. Chiles Research Institute, Providence Cancer Institute, Portland OR, USA

Even though blocking the interaction of NKG2A with its ligand HLA-E is a promising strategy to restore the function of cytotoxic T cells and induce tumor cell killing, several aspects of the biology of NKG2A+ CD8 T cells remain to be understood to fully benefit from this therapeutic approach. Here, we characterize the expression of NKG2A on tumor-infiltrating CD8 T cells (CD8 TILs) in patients with head and neck squamous cell carcinoma (HNSCC) and microsatellite-stable colorectal cancer (CRC). Our results show that NKG2A is primarily expressed by a population of tumor-reactive CD8 TILs identified by coexpression of CD39 and CD103 (DP CD8). NKG2A expression was stable and maintained during in vitro expansion. Interestingly, while TCR stimulation in presence of IL-15 and TGF-β are thought to be responsible for NKG2A upregulation, our in vitro results suggest that other factors might be necessary to induce its expression on naive CD8 T cells. Ex vivo analysis of NKG2A+ DP CD8 TILs by scRNAseq and flow cytometry revealed that those cells were activated, more differentiated, and displayed a cytolytic profile suggesting a role in tumor recognition and killing. Finally, we found that, even though there was a high degree of clonal overlap between NKG2A+ and NKG2A- DP CD8, some CDR3 TCR sequences were enriched in one versus the other subset. Consequently, HPV-reactive cells were preferentially enriched in NKG2A- DP CD8 TILs. Altogether, our results provide evidence that NKG2A is expressed by tumor-reactive CD8 TILs in HNSCC and CRC. However, NKG2A-inducing factor(s) remain to be identified and might provide new potential drug targets to bolster anti-tumor immune responses in cancer patients.

Presenter: Frank, Soveg

SARS-CoV-2 ORF8 regulates inflammation and viral replication

Soveg Frank, Mir M. Khalid, Irene P. Chen, Taha Y. Taha, Takako Tabata, Melanie Ott

The J. David Gladstone Institutes

The 29.9 kb genome of SARS-CoV-2 contains structural proteins, non-structural proteins, and accessory proteins. Although not required for replication, coronavirus accessory proteins can contribute to pathogenesis by manipulating host immune responses. Open reading frame 8 (ORF8) is a SARS-CoV-2 accessory protein containing a signal peptide and immunoglobulin domains. Previous studies have demonstrated ORF8 is secreted and binds to interleukin 17 receptor A (IL17RA). A now extinct variant of SARS-CoV-2 with a 382-nucleotide deletion (Δ384) in a region corresponding to ORF7b and ORF8 was associated with less severe COVID-19, suggesting ORF8 plays a role in viral pathogenesis. Here, we show a dual role for ORF8 in inducing proinflammatory gene expression and modulating viral replication. We find SARS-CoV-2 ORF8 is produced during infection in several cell lines. ORF8 is detected in cell culture supernatant in manner dependent on the signal peptide and is also present in the sera of mice infected with SARS-CoV-2. In immunoprecipitation experiments, we demonstrate ORF8 binds to the extracellular domain of IL17RA. Treatment of differentiated THP-1 cells with ORF8 results in dose-dependent production of proinflammatory cytokines. This induction is at least partially dependent on IL17RA as knockdown of IL17RA reduces ORF8-dependent gene expression. Surprisingly, when we infected cells with WA1 or Δ384 we found Δ384 has accelerated replication kinetics. Similarly, replication of a SARS-CoV-2 infectious clone with a stop codon in ORF8 was greater than the WT virus. Our data suggest that ORF8 acts as a proinflammatory IL17RA ligand while also attenuating viral replication. Whether both functions are linked is currently under investigation.

Presenter: Garcia Lopez, Laura

CD8 T cell IFN-γ differentiation in T. gondii requires NLRP3-dependent inflammasome-independent pathway

Laura Garcia Lopez, Angel Kongsomboonvech, Kirk D.C. Jensen

University of California Merced

Toxoplasma gondii is a protozoan parasite that is the causative agent of toxoplasmosis, an infection with a high prevalence worldwide. Part of the success of this parasite lies in the use of a membrane-bound vacuole, also named as a parasitophorous vacuole (PV) derived from the host, which allows the parasite to acquire nutrients and to avoid host defenses. One of the most important host defense strategies relies on the secretion of Interferon-γ (IFN-γ) by T cells. Previous studies using naïve transnuclear CD8 T cell to the endogenous T. gondii vacuolar resident antigen, TGD057 (T57) identified a novel pathway by which NLRP3 regulates CD8 T cells IFN-γ responses in an inflammasome cascade-independent manner. However, the exact signaling pathway from NLRP3 in the parasite-infected cell to IFN-γ production by the naïve CD8 T cell remains unclear. Using this approach, we report our ongoing efforts to elucidate this pathway including the investigation of type I IFNs, which may be initiated by NLRP3-dependent mitochondrial perturbation and STING activation in T. gondii infected cells. Taken together, the mechanism by responding CD8 T cells differentiate into IFN-γ producing cells has important implications for vaccine design and clearance of protozoan pathogens.

Presenter: Gauthier, Kelsey Sivick

AB308 is an Anti-TIGIT Antibody That Enhances Immune Activation and Anti-tumor Immunity Alone and in Combination with Other I-O Therapeutic Agents

Kelsey Sivick Gauthier, Dana Piovesan, Alejandra Lopez, Patrick G Schweickert, Ferdie Soriano, Ada Chen, Hema Singh, Xiaoning Zhao, Stephen W Young, Nigel Walker, Matthew J Walters

Arcus Biosciences, Inc

TIGIT (T-cell immunoreceptor with Ig and ITIM domains) is an inhibitory receptor expressed on natural killer (NK) cells, CD8+ T cells, CD4+ T cells and regulatory T cells (Tregs). On the surface of these cells, TIGIT competes with another receptor, CD226, for shared receptor ligands (mainly CD155) that are expressed by cancer and antigen-presenting cells. Binding of CD155 to TIGIT results in immune suppression through multiple mechanisms. When TIGIT is blocked, binding of CD155 to CD226 promotes immune activation and anti-tumor immunity. We describe the preclinical characterization of AB308, a humanized wild-type IgG1 anti-TIGIT antibody that is currently undergoing clinical evaluation. Here, binding of AB308 to TIGIT and inhibition of the TIGIT/CD155 interaction were evaluated in vitro. Functional assays were used to evaluate the immunomodulatory activity of AB308 alone or in combination with zimberelimab (anti-PD-1) or etrumadenant (a small molecule A2a/A2b adenosine receptor antagonist). Surrogate Fc-silent and Fc-enabled antibodies that recognize mouse TIGIT or PD-1 were leveraged to interrogate TIGIT biology in syngeneic mouse tumor models. We found that human tumor-infiltrating lymphocytes from a variety of cancer types expressed appreciable levels of TIGIT on relevant immune populations, including tumor reactive CD39+ CD103+ CD8+ T cells and Tregs. AB308 had a high binding affinity for human TIGIT, potently blocked the TIGIT-CD155 interaction, and induced Fcγ receptor (FcγR)-mediated signaling. In line with FcγRIII binding, AB308 also demonstrated the ability to induce NK cell-driven antibody-dependent cell-mediated cytotoxicity against TIGIT-expressing target cells. AB308 significantly increased IL-2 secretion by peripheral blood mononuclear cells activated with superantigen A, an activity that was further enhanced with zimberelimab. Blocking TIGIT with AB308 potently activated CD226 signaling in Jurkat T cells co-cultured with CD155-expressing cells, and combination of AB308 with etrumadenant in this system abrogated adenosine-mediated T cell suppression that occurred even in the presence of checkpoint inhibition. In mice, while combining Fc-silent or Fc-enabled anti-mouse TIGIT antibody with anti-PD-1 resulted in greater tumor growth inhibition than with anti-PD-1 alone, the activity of Fc-enabled anti-TIGIT was associated with intratumoral Treg depletion. In conclusion, AB308 is a potent and highly effective anti-TIGIT antibody. Concurrent blockade of multiple immune checkpoints has the potential to confer effective and durable responses in the treatment of cancer. The data presented here support the clinical use of AB308 and provides a rationale for combination with zimberelimab and adenosine pathway blocking agents such as etrumadenant and CD73 small molecule inhibitor, AB680.

Presenter: Gayer, Sarah

Il2 is a cell intrinsic repressor of IL-13 in in vitro differentiated Th2 cells

Sarah Gayer, Caroline Whitty, Ian Vogel, Jacob Freimer, Edward Marsh, Youjin Lee, Owen Jiang, ARum Yoo, Benjamin Lesch, Chang Liu, Hong Sun, Jeffrey Whitman, Alexander Marson, Sagar Bapat

UCSF

IL-2, an essential cytokine for T cell growth and proliferation in vitro and in vivo, signals via ligation of the IL-2 receptor (IL2R) on the cell surface of T cells. We conducted a pooled CRISPR screen to identify novel regulators of IL-13 expression in Th2 cells. Surprisingly, despite a high concentration of added IL-2 in the media, we discovered that Th2 cells with a disrupted Il2 locus had increased IL-13 expression. We validated this finding by targeted deletion of Il2 in Th2 cells and discovered that Il2 knockout cells have a two-fold increase in IL-13 expression relative to cells treated with an empty vector control. Importantly, the Th2 cells deficient in IL-2 maintained signaling through the IL2R, as measured by phosphorylation of STAT5. Further work will aim to elucidate mechanistic insights into how Il2 expression affects IL-13 levels within a Th2 cell. In summary, our data indicates a novel cell intrinsic role of Il2 in regulating IL-13 expression in Th2 cells.

Presenter: Gibbs, Lisa

Maternal Schistosomiasis Hinders Hematopoiesis Resulting in Aberrant B cell Maturation and Tolerance

Lisa Gibbs, Juan Marcos Oviedo, Keke C. Fairfax

University of Utah

Maternal helminth infections are a global public health concern that correlate with altered infant immune responses to childhood immunizations and infection. A mechanistic understanding of how maternal infection and inflammation alters the immune responses of offspring is lacking but is critical to decrease childhood morbidity and to understand the consequences of specific long-lived immunity defects. Using our model of maternal Schistosoma mansoni infection, we have shown that murine pups born to mothers chronically infected with Schistosoma mansoni have reduced responses to vaccinations, corresponding to what has been reported in humans. Additionally, these pups have reduced humoral immunity cell frequencies in the draining lymph node following Tetanus/Diphtheria immunization. To determine the origin of this humoral immunity defect, we began investigating the plasticity and functionality of progenitors of lymphoid cells critical for a protective humoral response. We found an increase in the common lymphoid progenitors (CLPs) in the bone marrow of pups from Schistosome infected mothers, but a decrease in transitional B cells and mature B cells in the periphery, indicating limitations during B cell maturation. When immunized with a Tetanus/Diphtheria vaccination, there is a significant reduction in expansion of these progenitors in comparison to controls from uninfected mothers coupled with a decrease in bone marrow B cells after positive selection, suggesting a more exclusive selection process or differential selective pressure than in pups from uninfected mothers that is confirmed by single cell RNAseq. We hypothesize that altered transcriptional regulation at the progenitor level caused by maternal Schistosomiasis is the mechanistic root of long-lived defects in humoral immunity to foreign antigens.

Presenter: Gonzalez, Michael

Phagocytic tumor macrophages are a distinct subset of tumor-associated macrophages that promote immune suppressive phenotypes and utilize oxidative metabolism.

Michael Gonzalez, Michael A Gonzalez, Daniel Lu, Maryam Yousefi, Carlos Briseno, Vivienne Watson, Sergey Novitskiy, Hong Zhou, Mike Tsai, Emily Ashkin-Loren, Chris Murray, Chi-Ming Li, Monte Winslow, Kristin Tarbell

Amgen Research and Stanford University

Macrophage abundance in tumors inversely correlates with clinical outcome in patients and hence are an attractive target for immunotherapy. A key feature of macrophages is their ability to phagocytose a milieu of pathogens, foreign bodies, cellular debris, and apoptotic cells in the body. However, precisely how phagocytosis shapes macrophage heterogeneity and function in solid tumors remains largely unknown. Here we utilized both an acute (cell transfer) lung tumor model and a novel epithelial-specific chronic (autochthonous/spontaneous) lung tumor model to study the phagocytosis of neoplastic cells by tumor-associated macrophages in vivo. Interstitial macrophages (IM) were found to be the most abundant phagocytic tumor (PHAT) macrophage in acute lung tumors, whereas alveolar macrophages (AM) represented the majority of PHAT macrophages in chronic autochthonous lung tumors. Both PHAT-AM and PHAT-IM had a mixed pro/anti-inflammatory phenotype marked by increased cell surface expression of antigen presentation (H-2k, H2-Ab1), immune checkpoint (PDL1), and costimulatory molecules (CD80, CD86) compared to non-PHAT macrophages in both acute and chronic tumors. Additionally, PHAT macrophages expressed significantly lower levels of proinflammatory proteins (IL6, IL1b, Tnf and Myd88) and increased expression of anti-inflammatory proteins (Tgfb1, Arg1). Single cell RNAseq revealed distinct cell clustering and expression trajectories of PHAT lung myeloid cells in autochthonous lung tumors indicative of an imprinted phenotype in response to phagocytosis of neoplastic cells. Additionally, we established a MonoMac phagocytic signature found to be enriched in human non-small cell lung cancer myeloid cells compared to matched adjacent normal tissue myeloid cells. Lastly, we identified oxidative phosphorylation (OXPHOS) as a key component of this signature and found significantly increased protein expression of OXPHOS genes and functional utilization of OXPHOS in both AMs and IMs in vivo and ex vivo. Together, our results identify PHAT macrophages as a distinct subset of macrophages that promote an immune suppressive phenotype in vivo and utilize OXPHOS metabolism.

Presenter: Greene, Trever

pDC exhaustion during infection: an underlying mechanism and its evolutionary benefit

Trever Greene, Yeara Jo, Monica Macal, Elina Zuniga

University Of California San Diego

Type I Interferons (IFN-I) are critical cytokines that protect against viral infections. While any cell can make IFN-I, plasmacytoid Dendritic Cells (pDCs) make higher quantities and more subtypes of IFN-I than any other cell type. However, following viral infection pDCs become “exhausted” losing their capacity to produce IFN-I. This phenotype is long-term in non-resolving viral infections and compromises host defense against secondary insult. It is intriguing that despite this apparent cost of pDC exhaustion this response is preserved across species and occurs in response to multiple types of viral infections. Through investigation of the transcriptional program of exhausted and non-exhausted pDCs we identified losses in pDC metabolic capacity that associate with pDC exhaustion. Mechanistically, we demonstrate restoration of a single repressed enzyme, lactate dehydrogenase B (LDHB), is sufficient to restore exhausted pDC function in vitro and in vivo. Furthermore, this same in vivo restoration enhanced infection-associated colitis. Therefore, our work identifies a novel mechanism for the regulation of interferon production in pDCs by LDHB, but also provides a potential explanation for the evolutionary benefit of pDC exhaustion in preventing immunopathology.

Presenter: Hagan, Cassidy

The influence of apoptotic cells on cancer metastasis

Cassidy Hagan, Annelise Snyder, Andrew Oberst

University of Washington

Apoptotic cell death is a common event within tumors, particularly following cytotoxic cancer therapy. Canonically, apoptotic cells induce anti-inflammatory, tolerogenic, and tissue reparative programs within tissues. Despite cell death by apoptosis being the primary goal of most cancer therapies, the impact these dying cells have on the tumor microenvironment, immunological landscape and ultimately cancer progression is only modestly characterized. Clinical correlations between high levels of apoptotic cell death and progression to metastatic disease exist in various types of cancer, yet it is not well understood how the programs induced by apoptotic cells influence the metastatic cascade. Using two distinct intravenous experimental metastasis models, B16.F10 melanoma and Met-1 breast cancer, we found that the presence of apoptotic cells increases experimental metastasis. This effect is reduced by blocking the apoptotic eat-me signal, phosphatidylserine, or by depleting phagocytic monocytes. As early as 6 hours we can detect a survival advantage of tumor cells coinjected with apoptotic cells. These data suggest that phosphatidylserine exposed on apoptotic cells engages phagocytic monocytes, which promote the early survival of circulating tumor cells. Ongoing project directions seek to further define other features of the apoptotic program influencing monocytes and supporting the survival of circulating tumor cells, ultimately with the goal of informing therapeutic strategies to combat the promotion of metastasis by apoptotic cells.

Presenter: Hammond, Elizabeth

Ineffective polarization characterizes CD4 T cell responses to persistent *Borrelia burgdorferi* infection

Elizabeth Hammond[1,2], Kim Olsen[2], Nicole Baumgarth[1,2,3]

[1]Graduate Group in Immunology, [2]Center for Immunology and Infectious Diseases,
[3]Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine
University of California Davis

Appropriate polarization of effector CD4 T cells is critical for the generation of effective immunity to pathogens. CD4 T cell polarization to infection with *Borrelia burgdorferi* (*Bb*), the spirochetal agent of Lyme disease, is considered to be Th1 dominant. *Bb*, however, establishes persistent infections in mice and humans, raising the questions about the immune response qualities that cause a failure of bacterial clearance. Here we re-examined effector CD4 T cell responses to *Bb* infection in C57BL/6 mice. Our studies showed that the signature genes of Th1 polarization (*Ifng* and *Tbx21*) were induced only transiently in activated lymph node, peripheral blood or spleen CD4 T cells early in *Bb* infection and only modest numbers of Tbet-expressing CD4 T cells were measurable by flow cytometry in long-term infection. This was not explained by enhanced polarization to other T effector types, Th2 or Th17, although early but transient polarization to Tfh was noted. Analysis of the skin, an important effector site, demonstrated that resident skin CD4 T cells, while increasing in response to infection, appeared largely non-polarized. Utilizing a novel I-A^b tetramer that identifies CD4 T cells specific for *Bb* arthritis-related protein demonstrated a similar lack of polarization. We conclude that infection with *Bb* fails to drive strong effector T cell polarization. Somewhat surprisingly, mice with total or T cell-specific genetic ablation of Tbet showed little difference in *Bb* tissue burden compared to control wild type mice. Weak induction of Th1 polarization is either insufficient to effectively control *Bb* burden, or perhaps more intriguingly, it may allow *Bb* to escape T cell immunity and establish persistence.

(Funding: Steven & Alexandra Cohen Foundation, Global Lyme Alliance, NIAID T32 AI060555)

Presenter: Hassan, Intisar

The role of temperature on macrophage functional activity in young and aged mice.

Intisar Hassan[1], Allison Strope[2], Satyajit Rath[3], and Jeannine M Durdik[1]

[1,2] University of Arkansas, [3]Indian Institute of Science Education and Research, Pune, India

Fever responses provide a survival advantage. Macrophages function under a range of temperatures experienced by the core and extremities and at the elevated body temperatures of fever. We examined the effect of temperature on the in vitro immune response of young and aged mouse macrophages. Macrophages isolated from young (4-6 months) and old (18-24 months) mice were activated with lipopolysaccharide (LPS) or IFN-γ or both. Stimulated mouse bone marrow derived macrophage (BMDM) and splenic macrophage (SM) cells from young mice show increased NO production and protein synthesis at 39°C compared to 37°C. At fever temperature, BMDM from aged mice had even higher levels of NO in response to LPS and IFN-γ than young mice. Stimulated SM from aged did not further increase NO or protein synthesis at 39°C over 37°C. On a per cell basis peritoneal resident macrophages (PRM) from aged mice produced less NO at both 37°C and 39°C compared to those from young mice. However, as the cell number of PRM present in aged mice is three to four times higher than young mice, the production of NO per mouse is higher in the aged PRM. PRM from young mice tested higher for protein synthesis under all conditions than at 39°C compared to 37°C. The unstimulated PRM from aged mice protein synthesis was higher than unstimulated young and showed some increase with LPS at 39°C. Adding LPS and IFN-γ to PRM, BMDMC and a mouse macrophage cell line [RAW264.7] increases the PER measured glycolytic rate in a NO dependent manner at an earlier time at 39°C compared to 37°C. Our findings suggest that increasing host body temperature can enhance macrophage function over a narrow range of temperatures and that the magnitude of this function is dependent upon the age and inflammatory state of the host and the local tissue environment.

Presenter: Hawkins, Reed

Suppressing androgen receptor activity in T cells promotes effective checkpoint blockade

Reed Hawkins, Xiangnan Guan, Fanny Polesso, Chaojie Wang, Archana Sehrawat, Susan E. Murray, George Thomas, Breanna Caruso, Julie N. Graff, Zheng Xia, Amy E. Moran

Oregon Health & Science University

Immune checkpoint blockade has revolutionized the field of clinical oncology, leading to durable anti-tumor immune responses in a range of solid tumors. However, in advanced prostate cancer patients, immunotherapy treatments have largely failed. Androgens have pleotropic functions in prostate cancer; they are potent drivers of cancer cell growth and can suppress T cell function and IFNγ production. In metastatic prostate cancer, intratumor androgen levels can remain high. Androgen deprivation therapy, the backbone of clinical care in prostate cancer, is classically administered to inhibit tumor cell growth. However, we postulated that this therapy also impacts non-malignant cells within the tumor microenvironment, including tumor-associated T cells. To test this hypothesis, we performed patient biopsy transcriptome profiling of individual leukocytes isolated from metastatic prostate cancer lesions resistant to androgen receptor inhibition and prior to treatment with PD-1 blockade. We reveal that inhibition of androgen receptor (AR) activity in CD8 T cells is critical for PD-1 pathway blockade, improving IFNγ expression and tumor regression. Notably, AR directly bound to *Ifng* and eviction of this transcription factor with a small molecule AR inhibitor or genetic knockout significantly increased cytokine production in CD8 T cells. Furthermore, we demonstrate that AR blockade sensitizes a non-prostate tumor mouse model to effective checkpoint blockade by directly enhancing CD8 T cell function. Together, our findings establish T cell specific AR activity as one mechanism of immunotherapy resistance in castration resistant prostate cancer and could be leveraged to improve immunotherapy outcomes.

Presenter: Hilbert, Zoë

Evolutionary arms races at the host-fungal interface

Zoë Hilbert, Nels Elde
University of Utah

Conflicts between mammalian immune systems and the microbes that infect them have driven countless "molecular arms races" across evolutionary history, the study of which can bring to light a unique understanding of host immunity and the forces that have driven adaptation of critical immune factors. Despite the successes of using evolutionary signatures to identify and study the nature of host-pathogen arms races with viruses and bacteria, little is known about similar conflicts between mammalian hosts and pathogenic fungi, which represent an important class of human pathogens. To examine whether mammalian hosts have engaged in such conflicts with fungal pathogens, we performed a phylogenetic screen: first identifying key host immune factors that recognize and engage with fungal cells and then performing phylogenetic analyses to look for signals of positive selection in multiple distinct mammalian lineages. This revealed differing patterns of adaptive evolution both at the gene-level as well as the species level, suggesting the possibility that fungi have differentially shaped the evolution of these genes in mammals. The vast majority of the positively selected genes identified through this approach are members of the C-type lectin receptor (CLR) family and other similar carbohydrate-binding immune factors involved in detecting and binding to microbial cell walls. Our preliminary work has demonstrated that positively-selected residues in these receptors are enriched in the ligand binding domains, and, in some cases, may directly correspond with residues thought to be involved in carbohydrate recognition, hinting at potential functional consequences of this sequence variation which our future work will aim to investigate. Together, these analyses not only underscore the importance of fungi in shaping the evolution of mammalian immune systems but also suggest a new role for diverse types of biological macromolecules beyond proteins in regulating the evolution of eukaryotic organisms, adding a new dimension to the possible battlefronts of host-microbe interactions.

Presenter: Horowitz, Nina

Novel Natural Killer Cell Therapies Can Infiltrate and Resist the Immunosuppressive Tumor Microenvironment

Nina Horowitz, John Hickey, June Ho Shin, Gail Snyder, Garry Nolan, John Sunwoo

Stanford University

Designing immunotherapies using insights from tissue-resident cells represents a novel method for enhancing trafficking into and retention within solid tumors. To this end, we developed methodology for differentiating peripheral blood natural killer cells (pbNKs) into cells resembling intraepithelial group 1 innate lymphoid cells (ieILC1s) in vitro and assessed their immunotherapeutic potential. We co-cultured irradiated epithelial tumor cells and pbNKs from healthy human donor blood to generate ieILC1-like cells. We assessed the differentiation using fluorescent flow cytometry and further profiled them using cytometry by time of flight (CyTOF) to obtain higher-dimensional data about their surface and intracellular phenotypes. We then quantified their cytotoxicity against a variety of target cell lines using the xCELLigence platform. Next, we grew three-dimensional tumoroids from single-cell suspensions of epithelial tumor cell lines in basement membrane extracts and added fluorescently labeled pbNKs and ieILC1-like NKs. We assessed their infiltration capacity into the tumoroids using confocal microscopy. Lastly, we stimulated the cells in the presence or absence of an overnight TGFβ exposure to determine the extent of TGFβ-mediated immunosuppression. ieILC1-like cells generated in vitro had comparable surface and intracellular phenotypes to ieILC1s in healthy tissue. They exhibited significantly increased cytotoxicity against multiple SCC cell lines and were also capable of antibody-dependent cellular cytotoxicity (ADCC), which we tested using the anti-epidermal growth factor receptor antibody cetuximab. Importantly, ieILC1-like cells efficiently infiltrated the tumoroids in a manner consistent with their tissue-resident phenotype and at higher rates than conventional pbNKs. These cells were also more resistant to TGFβ immunosuppression and produced significantly more IFNγ after stimulation than their conventional NK cell counterparts, despite being exposed to TGFβ overnight. Overall, our data show that ieILC1-like cells can be easily generated from pbNKs and provide a novel platform for adoptive cell therapy, as they exhibit strong natural cytotoxicity and ADCC against multiple targets. Most strikingly, ieILC1-like cells have an enhanced capacity for infiltration into solid tumoroids and seem to resist the immunosuppressive signals found within the solid tumor microenvironment. Future work will include in vivo quantification of tumor infiltration and efficacy as well as optimization of the co-culture platform to maximize differentiation, expansion, and cytotoxicity.

Presenter: Hu, Kenneth

Tumor, the wound that never heals? Use of gene program analysis to identify conserved cell-cell interactions between the healing skin wound and tumor settings

Kenneth Hu, Nicholas F Kuhn, Tristan Courau, Alexis J Combes, Bushra Samad, Matthew F Krummel

UCSF, ImmunoX Initiative

Tissues, both homeostatic and pathological, represent systems that are not well-mixed. Combined with the differing length scales of cell-cell communication, it becomes necessary to incorporate spatial information on top of single cell profiling. We turned to a spatial + temporal dissection of the healing skin wound, generating a scRNA-Seq dataset of the immune and non-immune compartments across 4 spatial and 4 temporal coordinates. We applied non-negative matrix factorization (NMF) to extract gene programs (i.e. collections of co-expressed genes) to describe the heterogenous Monocyte/Macrophage, fibroblast and other populations of the healing wound as an alternative to clustering. Critically, we were able to identify modules of these gene programs across cell types with similar space-time profiles, thus reframing the dynamic process of wound healing as phases of coordinated gene program activation. This information allowed us to predict several cell-cell signaling axes and up-regulated genes downstream of these signals across the timescale of the healing wound which we confirmed using in vitro experiments. We then identified several of these gene programs conserved between the Mono/Macs of the healing wound and those in a combination of tumor models. We combined our identified cell-cell interactions above with our wound-tumor gene program 'translation table', to make informed predictions on fibroblast-endothelial-macrophage interactions in distinct tumor models and support these predictions using imaging. We also verified correlation of expression of marker genes for an interacting fibroblast (Postn+, Lrrc15+) and macrophage population (Gpnmb+,Trem2+) in human tumor samples, suggesting this interaction is conserved across species and tissue context. The potential to describe the tumor microenvironment in terms of wound healing phases informs our future studies of crosstalk between the immune and non-immune compartments.

Presenter: Hughes, Erik

Transcriptional co-activator OCA-B/Pou2af1 drives Th1- and Th17-mediated autoimmune demyelination through CD4+ T cell antigen recall response

Erik Hughes, Heejoo Kim, Wenxiang Sun, Dean Tantin

University of Utah

The development of novel therapeutics that reduce disease burden and minimize immunosuppressive effects remains an unmet need in the multiple sclerosis (MS) therapeutic landscape. Using a mouse model of MS, experimental autoimmune encephalomyelitis (EAE), we have shown that the transcription factor Oct1 is necessary in T cells for autoimmune demyelination but is dispensable for baseline immune function and clearance of a neurotrophic virus. Here we use a recently developed conditional allele of Oct1's lymphocyte restricted transcriptional co-activator, OCA-B, to show that T cell-intrinsic loss of OCA-B results in protection from EAE in both chronic and relapsing-remitting mouse models. Notably, OCA-B deficient mice on the non-obese diabetic (NOD) background show specific protection to relapses, highlighting the potential to target OCA-B in new MS patients to limit disease progression after onset. Adoptive transfer EAE and recall response models suggest protection derives from a reduced ability of OCA-B deficient Th1 and Th17 cells to respond to previously exposed antigens. This reduced response is associated with decreased central nervous system (CNS) CD4 T cell infiltration and inflammatory cytokine production. Using a newly developed OCA-B reporter mouse, we identify CNS infiltrating CD4 T cells with a range of OCA-B expression during peak EAE disease. Interestingly, OCA-B expression is associated with blasting CD4 T cell populations suggesting OCA-BHi expressing populations might drive inflammation in the CNS. Cumulatively, these results highlight OCA-B as a promising therapeutic target to reduce MS pathogenesis while minimizing immune-suppressive effects.

Presenter: Hutchinson, Geoffrey

Engineering nanoparticles to focus T cell help to target antigen-specific B cells

Geoffrey Hutchinson, Mark Langowski, Mary Fontana, Neil King, Marion Pepper

University of Washington

The interaction between antigen-presenting cells and the T cell receptor (TCR) is fundamental to the selection and differentiation of antigen-specific CD4 T cells, B cells and the resulting antibody response. A variety of antigen-dependent factors determine which antigen-specific T and B cells go on to form germinal centers (GCs). However, several factors surrounding GC formation and the subsequent production of memory B and long-lived antibody-secreting plasma cells are still not fully understood. B cells largely recognize and bind surface epitopes often defined by a protein antigen's structure, causing them to engulf and process the antigen as short peptides. These linear peptide fragments derive from internally or externally positioned segments along the protein's amino acid chain and are then mounted on class II Major Histocompatibility Complexes (pMHC-II) for presentation to CD4+ follicular helper T cells (TFH). However, an abundance of antigen-presenting B cells must compete for limited help from TFH to enter GCs. Those that do later exit as long-lived plasma cells, and memory B cells. In theory, engineering the T cell epitopes within an antigen could improve availability of TFH and focus help to target B cells, influencing GCs and augmenting antibody responses. However, proteins are often sensitive and unamenable to changes in their interior making it difficult to independently study these factors without altering the external surface and unintentionally impacting responding B cells. To address this, we have engineered computationally designed protein nanocages packaging model antigens to manipulate and track the antigen-specific immune response while minimizing changes to the particle surface. This will allow us to test the impact of T cell epitope quantity and diversity on an antibody response while minimizing changes to B cell selection. We hypothesize that by adjusting these parameters, we can independently and simultaneously alter the selection and differentiation of B and T cells characterizing an adaptive immune response. Here, we show that even small changes in antigenic diversity can alter the antigen-specific antibody response and aim to define a proof-of-concept model to study a variety of immunological phenomena and inform vaccine design.

Presenter: Joag, Vineet

Sensing and alarm function of vaccine-elicited SIV-gag specific CD8 TRM in the reproductive mucosa of rhesus macaques

Vineet Joag, Clare Quarnstrom, James Michael Stolley, Andrew Soerens, Jason Schenkel, Kathryn Fraser, Vaiva Vezys, Eric Hunter, Pamela Skinner, Benjamin Bimber, Rama Amara, and David Masopust

University of Minnesota

TRM constitute a recently identified lymphocyte lineage that occupies non-lymphoid tissues (NLT) without recirculating. Experiments in mice demonstrated that TRM express their functions in the context of living tissues by triggering antiviral responses in neighboring cells, by promoting local stimulation of innate and adaptive immune cells, and by recruiting immune effectors to sites of TRM reactivation. Collectively this is referred to as a 'sensing and alarm' function and supports the view of TRM as both effectors and sentinel cells. We wished to broadly identify TRM functions in non-human primates (NHP). Here we applied a prime-boost-boost vaccine modality in rhesus macaques to generate abundant SIVgag-specific CD8 TRM in the female reproductive tract (FRT) and 14 other non-lymphoid tissues. To assess sensing and alarm function, macaques were challenged intravaginally with CM9 peptide from the SIV-gag protein and necropsied 24 or 48 hours later to assess mucosal and circulating immune parameters. CD8 TRM reactivation increased expression of CD69 and granzyme B in SIV-gag specific CD8 T cells throughout the FRT at 24h, in situ proliferation (Ki67 expression) by 48h, and rapid antiviral and IFN response gene expression in essentially all hematopoietic and non-hematopoietic cells by 24h. Upregulation of effector genes in CD8 T cells, CD4 T cells, NK cells, and ILCs peaked at 24h and persisted at 48h. CITE-Seq analysis revealed that 24h post CM9 challenge, mucosal CD4 T cells expressed various antiviral genes including HIV restriction factors, and had reduced expression of the HIV-co-receptor CCR5. Increased numbers of vaginal T and B cells coincided with increased expression of chemokines and VCAM-1 on endothelial and stromal cells in the FRT and a concomitant reduction in circulating T cells, B cells and SIV-env-specific B cells. These data demonstrate that vaccine-elicited CD8 TRM respond rapidly and trigger local activation and the recruitment of innate, cellular, and humoral immune responses to the site of antigen exposure.

Presenter: Kersten, Kelly

Spatiotemporal co-dependency between macrophages and exhausted CD8+ T cells in cancer

Kelly Kersten, Kenneth H. Hu; Alexis J. Combes; Bushra Samad; Tory Harwin; Arja Ray; Arjun Arkal Rao; En Cai; Kyle Marchuk; Jordan Artichoker; Tristan Courau; Quanming Shi; Julia Belk; Ansuman T. Satpathy; Matthew F. Krummel

University of California San Francisco

T cell exhaustion is a major impediment to anti-tumor immunity. However, it remains elusive how other immune cells in the tumor microenvironment (TME) contribute to this dysfunctional state. Here we show that the biology of tumor-associated macrophages (TAM) and exhausted T cells (Tex) in the TME is extensively linked. We demonstrate that in vivo depletion of TAM reduces exhaustion programs in tumor-infiltrating CD8+ T cells and reinvigorates their effector potential. Reciprocally, transcriptional and epigenetic profiling reveals that Tex express myeloid-related factors that actively recruit monocytes to the TME and shape their differentiation favoring antigen-presentation. Using lattice light sheet microscopy, we show that TAM and CD8+ T cells engage in unique long-lasting antigen-specific synaptic interactions that fail to activate T cells, but prime them for exhaustion, which is then accelerated in hypoxic conditions. Spatially resolved sequencing supports a spatiotemporal self-enforcing positive feedback circuit that is aligned to protect rather than destroy a tumor.

Presenter: Kim, Caleb

The unique TCR Cα domain regulates T-cell development, homeostasis, and responses to agonist peptide-MHC.

Caleb Kim, Heather Parrish, Michael Kuhns

University of Arizona

T-cells play a critical role in adaptive immune responses upon recognition of peptide-antigens presented in the context of MHC molecules (pMHC). They do this using the T-cell receptor (TCR), which is made up of extracellular, transmembrane and intracellular regions. The major building block of the extracellular regions are immunoglobulin (Ig) domains, which consist of two β sheets pressed together with a hydrophobic core. For example, the variable (V) regions of the TCRα and TCRβ chains consist of Ig-folds. Likewise, the TCRα constant region (C) consists of an Ig-fold. However, the TCRα C region (Cα) is missing the top β sheet of its Ig-like structure and instead has two loosely-associated top strands (C and F strands) on its surface. Previous results suggests that this evolutionarily conserved region mediates TCR multimerization and impacts signaling in vitro, but the functional significance of this region in vivo has yet to be characterized. In this study, we made transgenic OTII Rag1KO C57Bl/6 mice with or without mutations on the Cα C-strand to determine what the fitness cost is for mice bearing mutated Cα domain. Our results suggest that mutating this region increases TCR tonic signaling, positive and negative selection in the thymus, thymic output into the periphery, and homeostatic survival in the periphery. We interpret our data as evidence that the unique Cα domain plays a role in fine-tuning TCR signaling.

Presenter: Klawon, David

Antigen-specific suppression by regulatory T cells confers self vs. non-self discrimination during infection

David Klawon, Harikesh S Wong, Christine H Miller, Donald M Rodriguez, Matthew T Walker, Jaime L Chao, Bridgett K Ryan-Payseur, Nicole K Ganci, Ryan K Duncombe, Riley R Curran, Victoria Lee, Erin J Adams, Mark Maienschein-Cline, Nancy E Freitag, Ronald N Germain, Peter A Savage

University of Chicago

The T cell repertoire harbors both self-reactive CD4+ conventional T cells (Tconv) and Tconv cells poised to respond to pathogen-derived antigens. In the context of infection, it remains unclear how the immune system primes robust effector T cell responses to pathogen-derived peptides while restricting responses to self-ligands. We hypothesized that Foxp3+ regulatory T cells (Treg) selectively suppress self-reactive Tconv cells of matched antigen specificity during infection. To test this hypothesis, we analyzed CD4+ T cell responses to a natural I-Ab-restricted self-peptide ("C4 peptide") derived from the prostate-specific protein Tcaf3, which directs a fraction of antigen-specific T cells into the Treg lineage during thymic development. We challenged wild-type male mice with L. monocytogenes engineered to express the C4 peptide ("Lm[C4]"), establishing a setting of molecular mimicry in which C4 is expressed by both pathogen and host. Wild-type mice were protected from prostatitis following infection, demonstrating that the self-selected Treg-biased response to C4 is dominant over the C4-specific Tconv response triggered by Lm[C4]. Tetramer analysis, single-cell RNA/TCR sequencing, and in situ imaging revealed that C4-specific Treg cells did not prevent the initial activation of matched Tconv cells, but restricted the subsequent differentiation and persistence of C4-specific effector cells via a previously undescribed "rapid response" phenomenon in which C4-specific Treg cells were intrinsically poised to locally accumulate prior to antigen-matched Tconv counterparts. Lastly, we engineered mice specifically lacking C4 peptide expression in the thymus ("C4ΔTEC mice") to abolish skewing of C4-specific cells to the Treg cell lineage. Following infection with Lm[C4], C4ΔTEC mice developed overt prostatic T cell infiltration, indicating that bystander mechanisms of Treg-mediated suppression are insufficient to control C4-specific Tconv cells in this setting. Importantly, the altered T cell response to C4 had no impact on the Tconv cell response to the Lm-derived LLO peptide, demonstrating pMHC-II specificity of Treg suppression. Cumulatively, our findings reveal that self-specific Treg cells responding to an invading pathogen can swiftly and selectively control antigen-matched Tconv cells without impacting other pathogen-derived Tconv specificities, revealing a key role for antigen-specific Treg suppression in conferring self vs. non-self discrimination.

Presenter: Klebanoff, Sam

Using Machine-Learning-Assisted High-Throughput Flow Cytometry to Study Antigen-Specific Activation of Human T Cells

Sam Klebanoff, Peter Morawski, Mitch Fahning, Daniel Campbell

University of Washington

Infinity Flow is a recently developed technique (Etienne Becht et al. Sci Adv 2021) that utilizes machine learning to analyze a potentially infinite number of flow cytometry markers simultaneously on a single sample. This technique represents an extremely powerful tool for the characterization of human T cell subsets and phenotypes. Although it is known that T cell responses to most diseases are extremely heterogeneous, the majority of flow cytometry assays for studying T cells use at most 5-10 markers of activation/exhaustion/phenotype. By combining Infinity Flow with a standard T cell activation-induced marker (AIM) assay, we have the ability to capture the full complexity of human T cells in various disease contexts. Furthermore, by using UMAP dimensionality reduction to analyze the resulting data, we can identify extremely rare populations of activated T cells (0.03% of cells or lower). This allows us to stimulate PBMC samples with pools of individual peptides and identify T cells specific for these peptides without needing to perform tetramer enrichment beforehand. Finally, we've developed a barcoding strategy based on staining with four separate CD45 antibodies conjugated to different fluorophores. This strategy allows us to test up to 15 different samples simultaneously in our assay, further increasing both throughput and power to compare between samples/stimulation conditions. In summary, we have harnessed the power of Infinity Flow to create a new assay for study of human T cell activation, dubbed Infinity Multiplexed-Activation-Profiling (InfinityMAP). With InfinityMAP, we will be able to describe the complex heterogeneity of human T cell activation in a more complete and unbiased manner than would be possible with any standard flow cytometry AIM assay.

Presenter: Klement, John

PD-L1 suppresses type I interferon in myeloid cells to repress CTL recruitment to lung metastases

John Klement, Priscilla S. Redd, Chunwan Lu, Alyssa D. Merting, Dakota B. Poschel, Dafeng Yang, Natasha M. Savage, Gang Zhou, David H. Munn, and Kebin Liu

Medical College of Georgia at Augusta University

To evade immune clearance by cytotoxic T lymphocytes (CTLs), tumors express immunosuppressive checkpoints such as PD-L1. Blockade of these checkpoints has shown durable efficacy in a wide variety of human malignancies, and the mechanism underlying PD-L1 induction of immune suppression via CTL-expressed PD-1 has been extensively investigated. However, despite its utilization as a clinical biomarker for response to PD-1/L1 directed therapeutics, the contribution of tumor-expressed PD-L1 (tPD-L1) is incompletely understood. To investigate the molecular consequences of CTL engagement with tPD-L1, we generated PD-L1 knockouts in murine sarcoma, breast and colorectal cancer cell lines from multiple mouse strains with CRISPR/Cas9. Strikingly, tPD-L1 conferred no protection from CTL-mediated cytotoxicity in vitro and did not promote primary tumor growth in vivo. However, tPD-L1 facilitated the formation of lung metastases in both spontaneous and experimental models by limiting CTL recruitment. Single-cell and bulk RNA-seq of tumor-bearing lungs following tPD-L1 loss demonstrated enrichment of an interferon-stimulated gene signature, which was localized to myeloid cells. In vitro and in vivo experimentation revealed that tPD-L1 engaged myeloid PD-1 (mPD-1) to antagonize type I interferon (IFN-I) and STAT1 signaling, repressing CXCL9/10-driven chemotaxis of CTLs to lung metastases. Analysis of clinical datasets similarly revealed that the response to PD-1 blockade in human patients correlated with IFN-I signaling in myeloid cells. We propose a model in which tPD-L1 regulates CTL function and recruitment indirectly in the lung metastatic microenvironment through a tPD-L1/mPD-1/IFN-I/STAT1/CXCL9-10 axis.

Presenter: Kovacs, Michael A.

Meningeal lymphatic drainage promotes T cell responses against Toxoplasma gondii but is dispensable for parasite control in the brain

Michael A. Kovacs, Maureen N. Cowan, Katherine M. Still, Samantha J. Batista, Isaac Babcock, Lydia Sibley, Ish Sethi, and Tajie H. Harris

University of Virginia

Toxoplasma gondii is an intracellular protozoan parasite that causes chronic brain infection in a wide range of mammalian hosts. Animal studies have demonstrated that continuous T cell recruitment to the brain is necessary for parasite control. However, it has remained unclear how T cells outside the central nervous system sense and respond to brain-derived microbial antigen. Here, we test the hypothesis that the newly described meningeal lymphatic system promotes T cell immunity during T. gondii brain infection. We find that chronic brain infection is associated with significant expansion of parasite-specific T cells in the cerebrospinal fluid (CSF)-draining deep cervical lymph nodes. T cell activation at this site is correlated with parasite burden in the brain and peaks during the late stages of chronic infection. Flow cytometric analysis of CSF reveals a population of activated dendritic cells that is not present in the CSF of naïve mice. Mature dendritic cells are also mobilized in the meninges, specifically in areas where CSF protein can be sampled. Disrupting meningeal lymphatic drainage by ligating the collecting vessels leads to impaired T cell responses in the deep cervical lymph nodes. Surprisingly, in spite of reduced T cell activation and IFN-γ production at this site, T cell responses in the brain remain intact, likely due to ongoing antigenic stimulation in lymph nodes that drain non-central nervous system tissue. Overall, we provide evidence that meningeal lymphatic drainage supports robust parasite-specific T cell responses in the deep cervical lymph nodes. Nonetheless, we find that drainage of central nervous system material to this site is dispensable for host-protective T cell responses in the brain.

Presenter: Krabak, Cathleen

Mucosal barrier differences may contribute to altered T cell homeostasis in the B10.A mouse

Cathleen Krabak, Sedem Dankwa, Kerry Zhu, Sophia Sarafova

Davidson College

T-cells play a crucial role in adaptive immune response. CD4+ and CD8+ T-cells, two subtypes with distinct functions, are kept in a ratio greater than 1. This ratio is reduced and often inverted in B10.A mice when housed at a conventional mouse facility, such as Davidson College, but not when housed at a specific pathogen free (SPF) facility. Closely related C57Bl/10 mice (B10) do not experience housing-based fluctuations in CD4/CD8 ratio. In both strains the CD4 and CD8 T cells are produced at the expected ratio in the thymus, however that ratio inverts in the B10.A mice by the age of 6 to 9 months, more dramatically in the males than the females. The inversion of the ratio was not due to loss of CD4T cell, but a significant increase of CD8 T cell numbers and a more modest increase of CD4 T cell numbers in B10.A as compared to B10 mice. The intrinsic capabilities of T cells to be activated, proliferate, and perform their respective functions do not differ between B10 and B10.A mice, thus we started exploring extrinsic factors that could influence the abundance and stoichiometry of CD4 vs CD8 T cells in the periphery. Because commensal bacteria in the gut is a major difference between conventional and SPF facilities and can influence the homeostasis of peripheral immune cell homeostasis, we wanted to investigate the interaction between the microbiome and the gut associated lymphoid tissue (GALT) in the B10 vs. B10.A genetic backgrounds. Sequencing of the B10.A genome revealed several insertions in exons of the mucin 3 (muc3) and mucin 4 (muc4) genes, which prompted us to investigate the level of expression of mucins in the colon by qPCR. We found a significant decrease in B10.A muc2 and muc3 expression as compared to B10 mice, while muc1 and muc4 levels were very similar. Confirmation of these mucosal barrier differences by histology and Western blot is in progress. To determine whether the difference in mucin expression leads to impaired barrier function, we performed oral gavage with FITC-dextran and found no difference in the leakiness of the gut between the two strains. However, when we investigated the cytokine profile of the two mouse strains, we saw signs of chronic inflammation, which could contribute to dysregulation of T cell frequency and number. The B10.A mice produced significant amounts of IL-10, indicating a possible chronic inflammation issue, or skewing towards Th2 responses. In contrast B10 mice produced high levels of CXCL10, indicating a possible skewing to Th1 responses. We are in the process of confirming these differences at the expression level in the colon by qPCR. In conclusion we have revealed mucosal barrier differences in the B10.A mouse that may be contributing to altered T-cell homeostasis.

Presenter: Krishnamurty, Akshay

TGFß-dependent LRRC15+ myofibroblasts dictate the tumor fibroblast setpoint to promote cancer immunotherapy resistance

Akshay Krishnamurty, Minh Thai, Justin Shyer, Matthew B. Buechler, Yeqing Angela Yang, Rachana N. Pradhan, Amber W. Wang, Patricia L. Sanchez,
Yan Qu, Cécile Chalouni, Debra Dunlap, James Ziai, Lucinda Tam, Merone Roose-Girma, Zora Modrusan, Sören Müller, Shannon J. Turley**

Genentech

Single cell transcriptomics has led to the generation of large-scale atlases to understand cellular heterogeneity across both healthy and diseased tissues at the highest resolution. Such atlases have allowed for in silico inferences of fibroblast ontogeny and function, but without in vivo substantiation are limited in their utility to inspire novel fibroblast-directed therapies to improve disease outcome. In cancer, single-cell studies identified the emergence of a myofibroblast population, in both mice and humans, uniquely marked by a highly restricted leucine rich repeat containing protein, LRRC15. However, the molecular signals underlying development of LRRC15+ cancer associated fibroblasts (CAFs) and their direct impact on anti-tumor immunity remain uncharacterized. In a mouse model of pancreatic cancer, we provide in vivo genetic evidence that TGFßR2 signaling in healthy Dermatopontin (DPT)+ universal fibroblasts is essential for development of tumor-associated LRRC15+ myofibroblasts. Analysis of tumors from 159 patients across 13 indications revealed a conserved axis from universal fibroblasts to LRRC15+ myofibroblasts. This axis is the predominant driver of fibroblast lineage diversity in human cancers. Using a newly developed Lrrc15-Diphtheria toxin receptor knock-in mouse model that enables selective depletion of LRRC15+ CAFs, we show loss of this population markedly reduced total tumor fibroblast content and recalibrated the CAF composition towards universal fibroblasts. In turn, tumor regression in response to anti-PD-L1 immune checkpoint blockade and expansion of polyfunctional effector CD8 T cells were significantly augmented upon ablation. Collectively, these findings demonstrate that TGFß-dependent LRRC15+ CAFs dictate the tumor-fibroblast setpoint to promote tumor growth, suppress CD8+ T cell functionality, and limit responsiveness to checkpoint blockade. Development of treatments that restore the homeostatic fibroblast set-point by diminishing pro-disease LRRC15+ myofibroblasts may improve patient survival and response to immunotherapy.

Presenter: Kuenzli, Marco

I would walk five hundred miles – Iteratively stimulated T cells as tools to understand mechanisms of nonlymphoid recirculation

Marco Kuenzli, Andrew Soerens, Stephen D. O'Flanagan, Vineet Joag, Milcah C. Scott, Clare Quarnstrom, David Masopust

Center for Immunology, Department of Microbiology and Immunology, University of Minnesota

Memory T cell migration facilitates whole-body immunosurveillance and protection against recurrent infections and cancer. In the absence of inflammation, tissue resident memory T cells (TRM) patrol non-lymphoid tissues while effector and central memory T cells (TEM and TCM) are mostly restricted to the blood and the blood/lymph system respectively. Memory T cells that retain the ability to recirculate through all compartments are rare and poorly characterized. Thus, non-lymphoid tissue entry and egress requirements under homeostatic conditions are not well defined. Using a heterologous prime-boost immunization scheme and adoptive transfers, we've generated an abundant murine memory T cell population that has been exposed to 50 viral infections over the last 10 years. We termed these cells iteratively stimulated T cells (ISTCs). Parabiosis experiments revealed that ISTCs acquired pan-migration properties: they can be found in blood, various non-lymphoid tissues, lymph nodes, and thoracic duct lymph (TDL) of the naïve parabiont. ISTCs do not express CD62L in the TDL and cannot access lymph nodes via high endothelial venules (HEVs). However, ISTCs can be found in afferent lymphatics of non-lymphoid tissues suggesting that these cells take a detour to access the lymphatic system. Using single cell RNA-sequencing and in vivo CRISPR-Cas9 screening, we will utilize ISTCs as tools to further elucidate mechanisms of T cell migration which ultimately could improve targeted delivery of cellular immunotherapies.

Presenter: Kuhn, Nicholas F.

Single Cell Profiling of Skin Wound Healing Reveals Synchronized Waves of Fibroblast and Macrophage Subpopulations across Space and Time

Nicholas F. Kuhn, Kenneth H. Hu, Tristan Courau, Matthew F. Krummel

University of California, San Francisco

In their living environment metazoans are exposed to a multitude of biologically foreign agents, such as other metazoans, parasites, bacteria, and viruses, as well as physical injury, toxins, and noxious chemicals. The combination of these extrinsic factors with intrinsic factors, such as self-produced metabolic waste products, genome mutation rate, and aging, are sources of cell and tissue damage, which disrupt the natural homeostasis of a multicellular organism. Failure to re-establish a new homeostasis after such a disruption can lead to permanent tissue dysfunction, failure, and, ultimately, death. Coordination of and communication between diverse cell types is required to repair such insults, guaranteeing survival of multicellular organisms. To elucidate how such a process unfolds from insult-to-alarm-to-repair-to-aftermath, we profiled the cellular and transcriptional contributions of immune and non-immune cells over space and time during a mouse skin wound healing model. By combining (A) mass cytometry, a custom-made (B) spatial single cell RNA sequencing (scRNAseq) technique plus bioinformatic analysis pipeline, and (C) tissue clearing-enhanced 3D (Ce3D) microscopy, we present a proteomic and transcriptional space-time map of skin wound healing. Mass cytometry data highlights three different monocyte/macrophage (Mono_Mac) populations (early, intermediate, late) that successively inhabit the wound area. In more detail, the spatial scRNAseq data provides evidence that two distinct early Mono_Mac populations infiltrate the outside or the center of the wound, while intermediate Mono_Macs move towards the interior edge or center of the wound, and the late Mono_Macs never enter the original wound bed, but instead establish themselves at the exterior edge of the wound. Spatial scRNAseq analysis of non-immune cells identifies 5 distinct fibroblast populations during the wound healing process. All 5 fibroblast populations display distinct space-time profiles, however, 4 of them show nearly identical space/time occupation with the previously identified MAC populations: “early center”, “intermediate center”, “intermediate edge”, and “late exterior” fibroblasts.Identifying factors of cross-communication between MACs and fibroblasts throughout this coordinated differentiation trajectory that ends in healed skin and establishment of a new local tissue homeostasis will inform us how such events can be manipulated in pathological settings with high fibroblast and macrophage involvement, such as tissue fibrosis or desmoplastic tumor formation. The correlation of diverse fibroblast and Mono_Mac populations over space and time during the skin wound healing process, in combination with published observations of fibroblast-macropage crosstalk in different tissue settings, suggest an intimate coordination between these two cell types that is dependent on co-localization and timing. Our data set also suggests parallel differentiation trajectories of Mono_Macs and fibroblasts that are shared over space and time. Identifying factors of cross-communication between Mono_Macs and fibroblasts throughout this coordinated differentiation trajectory that ends in healed skin and establishment of a new local tissue homeostasis will inform us how such events can be manipulated in pathological settings with high fibroblast and macrophage involvement, such as tissue fibrosis or desmoplastic tumor formation.

Presenter: Labarta-Bajo, Lara

Immune-astrocyte crosstalk in the aged cerebellum

Lara Labarta-Bajo, Nicola J Allen

Salk Institute for Biological Studies

Average life expectancy within the last century is the longest humanity has ever experienced, however, the mechanisms driving the adaptation (or maladaptation) of our brains to aging are poorly understood. Astrocytes are cornerstone for the development, functionality, and plasticity of neurons during development and in adulthood, but their neuroregulatory role in aging remains mysterious. We recently devised a nuclear RNA sequencing (nRNAseq) protocol to profile Sox9-expressing astrocytes in the cortex, hippocampus, and cerebellum of 4-month-old (adult) and 2-year-old (aged) WT C57BL/6 mice that avoids the need for aged transgenic mouse lines or viral intervention. With that, we confirmed previous findings by showing that: 1) astrocytes acquire unique, regional transcriptional signatures in aging, that differ in the cortex, hippocampus, and cerebellum, and 2) we discovered that aged astrocytes acquire a type I IFN transcriptional signature in all brain regions analyzed, that is remarkably exacerbated in the cerebellum. Such inflammatory signature involved up-regulation of antigen presentation machinery genes, interferon-stimulated genes, as well as the effector T-cell chemo-attractant Cxcl10, which readily suggests crosstalk between astrocytes and the immune system in the aged cerebellum. Via single-nucleus RNAseq of Sox9+ astrocytes, we further found 4 transcriptionally distinct astrocyte subsets in the adult and aged cerebellum all of which acquired inflammatory signatures in aging, indicating that such response to aging is not driven by individual astrocyte fates, but rather, by all aged astrocytes in this brain region. Concomitant with the astrocyte phenotypes, nRNAseq of neurons in the cerebellum revealed downregulation of presynaptic and glutamate receptor subunit genes alongside a decrease in the levels of presynaptic VGLUT1 and AMPAR synapses in aged mice in association with profound deficits in fine motor coordination, balance, and alterations in gait. Overall, our findings suggest tri-partite crosstalk between astrocytes, the immune system, and neurons in the aged cerebellum that could have overarching implications for the synaptic decay that occurs in neurodegenerative disease.

Presenter: Lara, Heber

The Role of Tuft Cell-Expressed KIT in Intestinal Immunity and Regeneration

Heber Lara, Hung-An (Anna) Ting, Jakob von Moltke
University of Washington

The small intestinal (SI) epithelium has two vital roles: acting as a barrier against pathogens and absorbing nutrients. This single epithelial layer can rapidly adapt to a wide range of demands as is highlighted in the type-2 immune response. Acting as type-2 sentinels, tuft cells detect helminth and protist secretions and then release IL-25 to promote production of IL-13 and other canonical type-2 cytokines by group 2 innate lymphoid cells (ILC2). IL-13 then acts on the prolific crypt cells to induce tuft cell expansion and thereby complete a feed-forward circuit important to worm clearance. However, how IL-13 induces tuft cell hyperplasia remains unclear. Our data show that tuft cells express cellular KIT (cKIT/KIT) and that IL-13 signaling through IL-4RA is both necessary and sufficient to induce KIT. Why tuft cells express KIT remains to be elucidated. KIT is a receptor tyrosine kinase required for cell proliferation and survival in a variety of cell types. KIT activation has been linked to intestinal regeneration within another intestinal epithelial lineage, Paneth cells, but this signaling has not been studied in tuft cells. I hypothesize that IL-13 stimulated tuft cells utilize KIT signaling to promote their survival and broader epithelial regeneration.

Presenter: Leal, Joseph M.

A highly adaptable self-amplifying RNA vaccine platform drives potent immunity targeting SARS-CoV-2

Joseph M. Leal, Jesse H. Erasmus, Amit P. Khandhar, Jacob Archer, Malcolm S. Duthie, Darrick Carter, Steven G. Reed, Peter Berglund

HDT Bio

The coronavirus disease 2019 (COVID-19) pandemic, caused by infection with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has had a devastating impact on global health, particularly in developing countries with limited access to approved vaccines. Although mRNA vaccines have proven effective at preventing severe disease, stringent storage requirements have limited their impact in developing countries. Of further concern is continued emergence of variants that are less responsive to immune-mediated protection elicited by current vaccines. In response, we rapidly transitioned our initial Alphavirus-derived self-amplifying RNA vaccine construct saRNA-CoV2S, to encode the Beta variant of the SARS-CoV-2 spike (S) protein (sa-RNA-CoV2S.B.351). These saRNAs were formulated with lipid inorganic nanoparticles (LIONs) that were designed to enhance vaccine stability, delivery, and immunogenicity. Injection of both LION/saRNA-CoV2S and LION/saRNA-CoVS.B.351 in mice drove rapid local activation of antigen-presenting cells without the systemic activation and interferon response observed following injection of lipid nanoparticles (LNPs; like those used in approved mRNA vaccines). In mice and monkeys, LION/saRNA-CoV2S induced potent IFNγ T cell responses, neutralized SARS-CoV-2 at high titers, and induced IgG2a-biased antibody responses that persisted for at least 7.5 months. LION/saRNA-CoV2S can be lyophilized, and complexed material stored at 4-8 C for extended periods, enabling access to communities underserved by current mRNA vaccines. These data support LION/saRNA-CoV2S as a promising next-generation RNA vaccine candidate and highlight advantages over approved mRNA vaccines. Clinical trials of LION/saRNA-CoV2S are now underway in several countries.

Presenter: Lee, Madeline

Assessing the impact of COVID-19 severity on peripheral NK cells

Madeline Lee, Aaron Wilk, Ruoxi Pi, Giovanny Martinez-Colon, William Greenleaf, Catherine Blish

Stanford University School of Medicine

Severe COVID-19 induces strong alterations in the peripheral immune system. Some immune cell types take on a protective role in this disease, while others contribute to disease pathology. One cell type whose role in COVID-19 is not yet known is the natural killer (NK) cell. In order to better understand the NK cell response to this disease, we profiled NK cells and whole PBMC from donors across the COVID-19 severity spectrum using CyTOF, single-cell RNA sequencing, and single-cell ATAC sequencing. We found that severe COVID-19 induces strong activation of peripheral NK cells. Moreover, the activated NK cells in these patients downregulate surface expression, but not transcriptional expression, of the activating receptors DNAM-1 and NKG2D. As both of these receptors can be internalized upon ligation, we assessed expression of the ligands for these receptors on other peripheral immune cells and identified a significant increase in the expression of the ligands for NKG2D and DNAM-1 on the monocytes of severe COVID-19 patients. Collectively, our results suggest that monocytes may activate NK cells via the ligation of activating receptors in severe COVID-19.

Presenter: Lee, Victoria

The endogenous repertoire harbors self-reactive CD4+ T cell clones that adopt a T follicular helper-like phenotype at steady state

Victoria Lee(1,9), Donald Rodriguez(1,9), Nicole K. Ganci(3), Sharon Zeng(2), Jaime L. Chao(3,4), Matthew T. Walker(3), Christine H. Miller(1), David E. J. Klawon(3), Mary H. Schoenbach(2), Junting Ai(5), Domenick E. Kennedy(5,6), Mark Maienschein-Cline(7), Nicholas D. Socci(8), Marcus R. Clark(5), and Peter A. Savage(2)

(1) Interdisciplinary Scientist Training Program, University of Chicago, Chicago, IL (2) Department of Pathology, University of Chicago, Chicago, IL (3) Committee on Immunology, University of Chicago, Chicago, IL (4) Present Address: Department of Immunology, University of Washington, Seattle, WA (5) Section of Rheumatology, Department of Medicine and Gwenn Knapp Center for Lupus and Immunology Research, University of Chicago, Chicago, IL (6) Present Address: Drug Discovery Science and Technology, AbbVie, North Chicago, IL (7) Research Informatics Core, Research Resources Center, University of Illinois at Chicago, Chicago, IL (8) Bioinformatics Core, Memorial Sloan-Kettering Cancer Center, New York, NY (9) These authors contributed equally

The T cell repertoire of healthy mice and humans harbors self-reactive CD4+ conventional T (Tconv) cells capable of inducing autoimmunity. Using T cell receptor (TCR) profiling paired with in vivo clonal analysis of T cell differentiation, we identified Tconv cell clones that are recurrently enriched in non-lymphoid organs following ablation of Foxp3+ regulatory T cells. A subset of these clones were highly proliferative in the lymphoid organs at steady state and exhibited overt reactivity to self-ligands displayed by dendritic cells, yet were not purged by clonal deletion. These clones spontaneously adopted numerous hallmarks of T follicular helper (Tfh) cells, including expression of Bcl6 and PD-1, yet failed to produce common effector cytokines at baseline. The homeostasis of polyclonal Tconv cells displaying similar features was critically dependent on B cells. Our work identifies a naturally occurring population of self-reactive Tfh-like cells and delineates a previously unappreciated fate for self-specific Tconv cells.

Presenter: Leung, Gabriel

IL-7 and IL-7R regulate fetal macrophage establishment via survival and proliferation

Gabriel Leung, Clint Valencia, Brian Krum, Anna E. Beaudin

University of Utah

We have recently reported that IL7R signaling regulates fetal macrophage development, in addition to lineage commitment and development of lymphocytes. Specifically, IL7R deletion and pharmacological blockade during a defined fetal developmental window impairs the establishment of fetal macrophage populations in the liver, lung, brain, and epidermis. Having identified that IL7R signaling contributes to fetal macrophage establishment, we hypothesized that IL-7 is also required for fetal macrophage establishment and that IL-7 signaling activates developmental programs that facilitate macrophage development via proliferation and survival. Immunofluorescence images of cryosectioned E14.5 murine embryos revealed abundant co- localized IL7R and F4/80 expression in fetal liver, lung, brain, and skin. IL7R+ F4/80 expressing cells were also found in close proximity to IL-7+ cells, suggesting they participate in IL-7 signaling. Moreover, germline deletion of IL-7 also resulted in postnatal depletion of fetal-derived macrophage populations. Antibody blockade of IL-7R receptor during late gestation revealed tissue-specific deficits in survival and proliferation the day before birth. Specifically, liver and lung macrophages showed increased apoptosis as determined by Annexin V staining, while brain and epidermal macrophages were less proliferative, as determined by Ki67 staining. Here we have shown that IL-7, in addition to IL7R, regulates establishment of tissue-resident macrophages and that fetal blockade of IL7R signaling regulates establishment by impairing survival and proliferation during late gestation. Ongoing work seeks to define IL7/IL7R signaling pathways in developing macrophages as well as use genetic models to pinpoint the requirement for IL7R in fetal myeloid specification.

Presenter: Li, Jie

Cholesterol metabolism in macrophages as a potential regulator of breast cancer resistance to hormone therapy

Jie Li, Brian Ruffell

Cancer Biology PhD Program, University of South Florida

The leading cause of cancer related death among women is breast cancer, with about 80% of patients presenting with estrogen receptor (ER) positive disease. For these patients, hormone therapies have been widely used to modulate estrogen levels or ER signaling; however, almost 30-40% of patients will experience resistance to therapy. Therefore, new therapies to prevent and treat hormone therapy resistance are urgently needed. Tumor-associated macrophages (TAMs) comprise a high proportion of immune cells in breast tumors and have been shown to promote resistance to chemotherapy in preclinical models. However, the role of TAMs in ER+ breast cancer and resistance to hormone therapy is unknown. Here we find that the human monocytic cell line THP-1 promotes ER+ breast cancer cell line MCF7 growth and resistance to tamoxifen without affecting the triple negative breast cancer cell line MDA-MB231, both in monolayer and as 3D spheroids. Depleting macrophages in E0771 tumors through anti-colony stimulating factor 1 (CSF-1) administration further reduces tumor growth in response to fulvestrant treatment in ovariectomized mice. Mechanistically, MCF7 growth and hormone therapy resistance occurred through activation of ER alpha, as evidenced by increased ER translocation into nucleus. Importantly, THP-1 cells can also transfer LDL as a source of cholesterol to cancer cells, suggesting that this may serve as a precursor for the intratumoral synthesis of estrogens. Our study highlights a mechanism of extrinsic resistance to endocrine therapy through cancer cell and TAMs interactions that could indicate new therapeutic approaches in breast cancer.

Presenter: Liao, Jingwen

The Role of the SWI/SNF Complex in Macrophage Inflammatory Response

Jingwen Liao
Salk Institute

Macrophages are innate immune cells that are highly dynamic and attuned to local signaling. In response to invading pathogen or stimulation in vitro via bacterial membrane component LipidA, macrophages adopt M1 polarization and activate inflammatory response genes. In contrast, macrophages can also adopt M2 polarization, which promotes wound healing and maintains tissue homeostasis. Given the versatility, macrophage transcriptional program needs to be tightly regulated. Hyper and prolonged inflammation would lead to tissue damage and a diverse range of morphological consequences that include sepsis, pulmonary fibrosis, and auto immune diseases. The chromatin remodeling SWI/SNF complex is a known regulator of macrophage inflammatory response. Its ATP-dependent catalytic subunit can modulate local chromatin accessibility via nucleosome sliding and eviction. Previous studies have found this catalytic activity critical for proper activation of select inflammatory response genes, but its genome wide involvement in the broader inflammatory response remains elusive. To better understand how the SWI/SNF complex regulates macrophage inflammatory response, we used in vitro differentiated BMDM (bone marrow derived macrophages) as a model system and profiled the canonical and predominant SWI/SNF complex that contains Arid1A using ChIP-Seq. At baseline, Arid1A colocalizes with lineage determining transcription factor Pu.1, corroborating its role in priming the chromatin landscape during development. In response to LipidA, on the other hand, Arid1A rapidly relocalizes to regulatory elements near inflammatory response genes that are also bound by transcription factors from the NFκB, AP1, and STAT family. Their binding coincides with chromatin opening, H3K27 acetylation, and nascent transcription, all of which are features of transcriptional activation. In addition, Arid1A relocalizes to enhancers within the first hour of stimulation but does not reach promoters until after four hours of stimulation. This suggests the SWI/SNF complex is involved is a well-orchestrated sequence of events that are kinetically regulated following stimulation. To further dissect the connection between the SWI/SNF complex and transcription activation of inflammatory response genes, we used a small molecule inhibitor for Arid1A to disturb SWI/SNF function. Inhibition of Arid1A leads to global loss of Arid1A binding and reduced chromatin accessibility at select sites. As a result, a large set of inflammatory response genes fails to be activated to maximum level. This supports our hypothesis that the SWI/SNF complex plays a broader role in macrophage activation than previous demonstrated, potentially through establishing chromatin accessibility and collaborative binding with stimulus responsive transcription factors.

Presenter: Lopez, Diego

Consequences of Maternal Immune Activation (MIA) on Neonatal Lung ILC2 Establishment, Function, and Airway Hyperresponsiveness

Diego Lopez, Aleah Griffin, Anna E. Beaudin

University of Utah School of Medicine

Our lab has previously identified a developmentally-restricted hematopoietic stem cell (drHSC) that only exists in the perinatal period and specifically gives rise to innate-like lymphocytes during development. The identification of a developmentally-limited cell of origin for innate-like immune cells that persist across the lifespan defines a "critical window" for immune development, in which the phenotype of the adult immune system can be shaped by extrinsic inputs. To test how developmental perturbation during this critical window drives immune dysfunction, we examined underlying changes to lung type-2 innate lymphoid cells (ILC2s) and susceptibility to airway hyperresponsiveness following maternal immune activation (MIA) with poly (I:C). ILC2s are a relatively recently identified subset of innate-like lymphocytes that make up the majority of ILCs in the lung, and are an important source of IL-5 and IL-13 cytokines upon parasitic infection or allergen-induced airway inflammation. ILC2s colonize the lung as early as embryonic day (E)17.5 and are believed to be derived, at least in part, from fetal precursors during embryonic development. Here we demonstrate that induction of MIA via a single low-dose injection of poly (i:c) at mid-gestation altered the establishment of an ILC-committed progenitor, the common helper innate lymphoid progenitor (ChILP), increasing both cell number and frequency of ChILPs both one-day and three-days post MIA. MIA also enhanced ILC2 proliferative capacity during early postnatal lung development, resulting in a robustly expanded ILC2 compartment at postnatal day (P)14 that persisted well into adulthood. Importantly maternal, but not neonatal, TLR3 signaling was required for ILC2 expansion at P14, suggesting maternal inflammation as the driver of postnatal ILC2 expansion and not direct pIC-TLR3 signaling in offspring. Concomitant with a greater inflammatory immune cell profile, lung ILC2s from MIA-treated offspring also exhibited a hyperactive profile: in-vitro stimulation yielded higher production of IL-5 and IL-13, cytokines important for eosinophil recruitment, activation and goblet cell hyperplasia during allergic airway inflammation. Furthermore, an expanded and hyperactivated ILC2 compartment postnatally led to a remodeling of the lung immune landscape: in addition to expanded ILC2s, we also observed expansion of B-, T-, NK-, NKT-cells, alveolar macrophages and eosinophils. To determine if observed alterations to the lung immune profile could be attributed to MIA-induced changes to the lung microenvironment, we profiled the expression of 25 distinct lung cytokines across P3-P9. Surprisingly, lung cytokine levels remained relatively static across early lung development in MIA or saline conditions, suggesting that observed changes to ILC2 proliferation and cytokine production are cell-intrinsic. Furthermore, ILC2s were the first immune population to show significant alterations in cell number in response to MIA postnatally, with increased cellularity observed 3-days prior to any significant difference in any other profiled immune cell population. Ongoing experiments are now focused on examining how altered ILC2 functionality, as a result of MIA, may underlie susceptibility to allergic asthma in response to secondary acute house dust mite (HDM)-induced airway hyperresponsiveness. Altogether, our data suggest MIA imparts lasting changes in the postnatal lung immune landscape by perturbing the establishment of committed ILC progenitors in the fetal liver, thereby altering the production of neonatal lung ILC2s. Expanded ILC2s are poised to hyperactively respond to secondary immune activation following prenatal inflammation, which may then contribute to greater allergic airway hyperresponsiveness.

Presenter: Lyons-Cohen, Miranda

Site-Specific Immunity and Cellular Clustering Regulate Th2 Differentiation

Miranda Lyons-Cohen, Miranda R. Lyons-Cohen, Michael Y. Gerner

University of Washington

Th2 cells are critical for protection against parasitic helminths, but can also drive inappropriate inflammation during allergy and asthma. Substantial efforts have gone into identifying the early mechanisms of Th2 differentiation in lymph nodes (LNs), yet how Th2 cells are generated in tissue context remains poorly understood. Here, we used multiplexed quantitative imaging to investigate the early processes involved in the generation of Th2 immunity within skin-draining lymph nodes (LNs) after cutaneous administration of the allergen protease Papain with OVA. We found that Papain immunization induced extensive early activation and ICAM1-mediated clustering of OVA-specific OT-II cells, as well as of endogenous CD4 T cells. These clusters were primarily localized at the T/B border and in association with migratory type-II dermal dendritic cells (DC2s). T cells within the clusters were characterized by high levels of TCR signaling, IL-4 mRNA and Gata3 expression, as well as phospho-STAT5 (pSTAT5) and pSTAT6 staining, together suggesting robust Th2 differentiation within dedicated LN microenvironments. Surprisingly, generation of these Th2 microenvironments was dependent on the specific site of cutaneous immunization. Papain administration in the footpads elicited significantly reduced T cell clustering and Th2 differentiation as compared to all other tested cutaneous sites, suggesting site-specific responses in the generation of Th2 immunity. Although T cells underwent relatively equivalent proliferation in all sites, T cells within footpad draining LNs exhibited reduced levels of IL-4 mRNA and Gata3 expression, IRF4, BATF and pS6 expression, suggesting diminished overall activation and Th2 differentiation. Consistent with this, mRNA-sequencing analysis of antigen-bearing migratory DC2s isolated from different LNs revealed distinct transcriptional and phenotypic profiles associated with cellular maturation and co-stimulation. Collectively, we identify dedicated microenvironments within LNs that support Th2 differentiation, and reveal that formation of these microenvironments is context-dependent, with the site of skin immunization being a critical factor in the generation of type-2 immunity.

Presenter: Mallajosyula, Vamsee

High-resolution epitope mapping identifies decoupling of the CD4+ and CD8+ T cell peak responses after mRNA vaccination

Vamsee Mallajosyula, Fei Gao, Scott D. Boyd, Kari C. Nadeau, Bali Pulendran, Mark M. Davis

Stanford University

It is critical to evaluate the T cell responses at single-epitope resolution after vaccination given their important role in controlling disease after SARS-CoV-2 infection. We probed a total of >275 blood samples collected from vaccinated volunteers with timepoints ranging from pre-vaccination up to 4 months after the first dose using pMHC "spheromers", a new platform we recently developed for better ex vivo characterization of αβ T cells. The spheromer stains T cells with high-sensitivity and captures a significantly broader T cell receptor (TCR) repertoire than the current standards (tetramer and dextramer). Overall, BNT162b2 vaccination elicited robust CD8+ and CD4+ T cell responses across all volunteers, likely contributing to its remarkable efficacy. The median frequency of CD8+ T cells for the most dominant epitope (S691) at the nominal peak (day 42) was 7.76%. In contrast, the peak response for CD4+ T cells occurred earlier at week-one post boost (day 28) with a median frequency of 12.24% for the dominant epitope (S191). Finally, we also evaluated the impact of hybrid immunity. Intriguingly, while prior infection had almost no effect on the CD4+ T cell response induced upon vaccination, we observed a significant decrease in the frequency of CD8+ T cells. These results can inform vaccination strategies going forward.

Presenter: Maltbaek, Joanna H.

ABCC1/MRP1 exports cGAMP and modulates cGAS-dependent immunity

Joanna H. Maltbaek, Jessica M. Snyder, Daniel B. Stetson

University of Washington

The DNA sensor cyclic GMP-AMP synthase (cGAS) is important for antiviral and antitumor immunity. cGAS generates cyclic GMP-AMP (cGAMP), a diffusible cyclic dinucleotide that activates the antiviral response through the adapter protein Stimulator of Interferon Genes (STING). cGAMP is negatively charged and cannot passively cross cell membranes, but recent advances have established a role for extracellular cGAMP as an "immunotransmitter" that can be imported into cells. However, the mechanism by which cGAMP exits cells remains unknown. Here, we identify ABCC1/MRP1 as an ATP-dependent cGAMP exporter that influences STING signaling and type I interferon production. We demonstrate that ABCC1 deficiency exacerbates cGAS-dependent autoimmunity in the Trex1-/- mouse model of Aicardi-Goutières syndrome. These studies identify ABCC1-mediated cGAMP export as a key regulatory mechanism of the cGAS-STING pathway.

Presenter: Matter, Aubry

A New Spontaneous Model of Multiple Sclerosis Requiring IFN-γ Deficiency

Aubry Matter, Denny Liggitt, Joan Goverman

University of Washington Immunology

Multiple Sclerosis (MS) is a demyelinating autoimmune disease of the central nervous system (CNS) that has been studied using the animal model Experimental Autoimmune Encephalomyelitis (EAE). Our lab previously generated an MHC class-I restricted TCR-transgenic model referred to as "8.8" mice to investigate the role of myelin-specific CD8+ T cells in EAE. We demonstrated that 8.8 T cells exacerbate CD4 T cell-induced EAE in an IFNγ-independent manor. In contrast, EAE induced directly in 8.8 mice by infection with vaccinia virus requires that the 8.8 T cells express IFNγ to develop disease. Unexpectedly, we observed that 8.8 mice on an IFNγ KO background develop spontaneous EAE (SEAE) at a high incidence while SEAE is very rare in 8.8 IFNγ +/+ mice. This SEAE recapitulates aspects of MS not seen in other models: it is female-biased with an age of onset corresponding to the typical age of onset of MS in women, and the pathology is focused primarily in the brain. Interestingly, the predominant lymphocyte in lesions are B cells, which are the main presenters of the myelin epitope. Further elucidation of the role between IFNγ & B cells in SEAE is important for better understanding the role of B cell-depleting therapies.

Presenter: Maxwell, Matthew

Loss of the ARID1A Tumor Suppressor Activates a STING-Type I Interferon Signaling Axis that Promotes Anti-Tumor Immunity

Matthew Maxwell, Marianne Hom, Diana Hargreaves

The Salk Institute for Biological Studies

ARID1A is a core protein subunit of the mammalian Switch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex and among the most frequently mutated tumor suppressors human cancers. Recently, checkpoint blockade immunotherapy clinical trials have identified ARID1A mutations as enriched among patients who respond favorably to immunotherapy in a diverse array of solid tumor types in a manner that is independent of microsatellite instability and tumor mutational burden. Thus, the molecular mechanisms that dictate improved immunotherapy responsiveness in ARID1A mutant tumors remain unclear. To investigate the mechanisms underlying improved immunotherapy response in ARID1A mutant tumors, I developed ARID1A deficient melanoma and colon cancer murine tumor models that recapitulate anti-tumor immunity phenotypes such as increased CD8+ T and NK cell infiltration and functionality observed in ARID1A mutant human cancers. Leveraging these ARID1A deficient pre-clinical tumor models and human tumor RNA-seq data, I discovered that ARID1A deficient cancers show prominent upregulation of an immunogenic Type I interferon (IFN) gene expression signature that includes interferon stimulated genes known to mediate cytotoxic immune cell recruitment and activation. Mechanistically, we have demonstrated that the ARID1A deficient Type I IFN gene expression signature is driven by a non-canonical cytosolic DNA sensing pathway that is dependent on STING and TBK1 but not the predominant mammalian cytosolic DNA sensor cGAS. Moreover, we demonstrate that STING and Type I IFN signaling but not cGAS are required for ARID1A deficient anti-tumor immunity. The source of cytosolic DNA in ARID1A deficient cancer cells appears to be driven by a defect in DNA replication stress that generates large quantities of single stranded DNA (ssDNA) and micronuclei in the cytosol which mimics a DNA viral infection and drives ARID1A mutant anti-tumor immune response. These findings represent novel molecular mechanisms underlying increased tumor immunogenicity in ARID1A-mutant cancers and provide a possibility for combining ARID1A-mutation status with Type I IFN gene expression signature status from tumor biopsies to determine which patients may benefit most from immunotherapy.

Presenter: Miranda, Nadia

Imaging the lung microenvironment during Coccidioidomycosis

Nadia Miranda, Anh Diep, Susana Tejeda-Garibay, Katrina K Hoyer

UC Merced

Valley fever is a respiratory disease caused by the fungal pathogen Coccidioides. Very little is known about how the fungus interacts with the immune system and the lung microenvironment, hampering vaccine and therapy development. There is a critical need to identify which immune cells are interacting with the fungus in the lungs and how these cells control infection. Granulomas form within the lung to control infection, but the formation, maintenance, and molecular and immune players in these processes are largely unexplored. Granuloma cell mass can be identified in X-rays and MRI scans only to be misdiagnosed for tumors, until a lung biopsy is analyzed. To investigate immune cells present in Coccidioides granulomas, we utilized tissue immunohistochemistry via fixed-formalin paraffin-embedded (FFPE) and fresh frozen embedded samples to assess immune cell and Coccidioides interactions. Various labeling methods have been investigated using infected mouse lungs to optimize procedures before switching to patient samples. Hematoxylin and eosin (H&E) staining combined with periodic acid–Schiff (PAS) identifies Coccidioides spread within the lung following fungal inhalation in mice. Lung imaging challenges include the presence of endogenous peroxidases that cause non-specific antibody binding. Antigen retrieval following paraffin embedding, have now been optimized to fit the lung and specific to the antibody that is being used. Here we highlight various troubleshooting methods with successful immunofluorescence FFPE lung imaging.

Presenter: Morawski, Peter

Cutaneous CD4+ T cell subsets promote distinct outcomes in human keratinocytes and fibroblasts

Peter Morawski, Peter A. Morawski, Hannah DeBerg, Mitch Fahning, Caroline Stefani, Adam Lacy-Hulbert, Iris K. Gratz, Daniel J. Campbell

Benaroya Research Institute

Epithelial, stromal, and immune cells in the skin cooperate to ensure appropriate response to pathogens and environmental damage. Recent single cell transcriptional analyses of healthy and fibrotic human skin revealed a wide range of phenotypes that arise in response to distinct cytokine milieu. Cutaneous T cells promote host defense and the subsequent resolution of inflammation and repair of damaged skin through the production of cytokines, but the precise mechanisms T cells use to impact the differentiation and function of skin cells involved in inflammatory and tissue-repair responses remain understudied. To assess the functional capacity of cutaneous lymphocytes we isolated skin-tropic and skin-resident T helper cells including a novel population of pro-fibrotic migratory tissue resident memory cells we recently discovered. We measured both quantitative T cell cytokine production following TCR stimulation, and the impact of T cell derived cytokines on human keratinocyte and fibroblast transcriptional profile and morphology. We find that phenotypically and functionally distinct circulating and skin-resident T cell subsets have the capacity to promote discrete cytokine-dependent transcriptional and cellular outcomes in the skin including the induction of metabolic, proliferative and inflammatory gene profiles. Thus, cutaneous T cells support host-protective and tissue-repair responses through direct activity on keratinocytes and dermal fibroblasts.

Presenter: Morris, Gerald

Dual receptor T cells mediate effective anti-tumor immune responses via increased recognition of tumor antigens

Gerald Morris, Hyun J. Jang, Calvin K. Lee, Christine Caron, Burhan Jama, Jack D. Bui

University of California San Diego

Recent discovery of the unexpectedly large portion of T cells naturally co-expressing 2 T cell receptor (TCR) clonotypes due to allelic inclusion of TCRα genes during thymopoiesis prompts examining the role of dual TCR cells in immune functions. Using our novel TCRα-reporter transgenic mice, which enable unambiguous identification of single- and dual-TCR cells, we tested the role of dual TCR cells in anti-tumor immune responses against immune-responsive syngeneic 6727 sarcoma and immune-resistant B16F10 melanoma. Dual TCR cells were specifically increased among tumor-infiltrating lymphocytes (TILs) in both models, and disproportionately activated in immune-mediated elimination of 6727, demonstrating a selective advantage in responding to tumors. Absence of dual TCR cells impaired immune response to B16F10 but not 6727, suggesting that dual TCR cells may be more important for responses to poorly immunogenic tumors. We identified a selective advantage of dual TCR cells in responses to B16F10-derived neoantigen peptides in vitro, providing a mechanistic basis for their increased anti-tumor reactivity. These results discover a novel role for dual TCR cells in protective immune function and identify these cells and their TCRs as a possible resource for anti-tumor immunotherapy.

Presenter: Mroz, Nicholas M.

ILC2s limit cortical damage and neuronal hyperexcitability after ischemic stroke

Nicholas M. Mroz, Rafael Taeho Han, Nathan A. Ewing-Crystal, Madelene W. Dahlgren, Ilia D. Vainchtein, Leah C. Dorman, Jeanne T. Paz, Anna V. Molofsky, Ari B. Molofsky

University of California, San Francisco

Adult brain injuries such as ischemic stroke are a leading cause of morbidity and mortality worldwide due to the limited capacity for adult brain regeneration, and there are no effective therapies to improve outcomes after stroke. Injuries to the central nervous system (CNS) initiate a sterile inflammatory response that recruits peripheral immune cells, such as macrophages and neutrophils, which can in turn mediate secondary damage. In contrast, type 2 immune responses are protective after stroke and primarily act during the resolution phase. The type 2 cytokines Interleukin-33 (IL-33) and IL-13 are protective after CNS injury, but the mechanism(s) for this effect, including the sources and targets of these signals, are poorly defined. Our group has identified a prominent population of group 2 innate lymphoid cells (ILC2s) in the meningeal membranes that surround the brain. ILC2s are tissue-resident innate lymphocytes that participate in wound healing and tissue remodeling processes and have recently been shown to impact brain physiology and function. ILC2s are robustly activated by IL-33 and produce IL-13 when activated, raising the possibility that they may participate in beneficial type 2 immune responses to brain damage. Using the photothrombotic (PT) mouse model of ischemic stroke, we found that ILC2s in the meninges activate, expand, and limit cortical damage and corticothalamic hyperexcitability after injury in a partially IL-33 dependent manner. We also found that ILC2s modulate microglia and meningeal macrophage populations and inhibit excessive neutrophil accumulation after stroke. In addition, we observed that meningeal ILC2s closely localize to IL-33-expressing fibroblasts that can support their survival in vitro. Our working model is that IL-33 released from meningeal niche fibroblasts activates ILC2s to produce IL-13 and regulate microglia and infiltrating myeloid cells, thereby promoting recovery from CNS injury. Future work will determine the mechanism through which meningeal ILC2s alter cortical outcomes after stroke, as well as the source of IL-33 that activates ILC2s in this context.

Presenter: Nadjsombati, Marija

A single genetic locus regulates mouse strain-specific tuft cell differentiation and innate type 2 immunity in the small intestine.

Marija Nadjsombati, Marija S. Nadjsombati, Natalie Niepoth, Lily Webeck, Andrés Bendesky, Jakob von Moltke

University of Washington

Epithelial tuft cells are critical initiators of the small intestinal type 2 immune response as they are ideally positioned to monitor the intestinal lumen and signal to immune cells in the underlying tissue. During helminth infection or protist colonization, tuft cell-derived IL-25 activates group 2 innate lymphoid cells (ILC2s) in the lamina propria. IL-13 from ILC2s signals on intestinal stem cells promoting their differentiation into tuft cells, thereby driving tuft cell hyperplasia and completing a feed-forward tuft-ILC2 circuit. The importance of this circuit has been demonstrated in numerous contexts, but how epithelial progenitors become tuft cells remains poorly understood. Here we report a novel genetic locus that regulates tuft cell differentiation and the threshold of tuft-ILC2 circuit activation. C57BL/6J mice treated with succinate, a known intestinal tuft cell ligand, develop tuft cell hyperplasia whereas Balb/c mice do not. Balb/c mice can respond to succinate if first treated with recombinant IL-25, suggesting all components of the succinate-sensing tuft-ILC2 circuit are functional, yet differentially regulated between the two strains. This regulation is not dependent on the microbiome and can be recapitulated in epithelial organoids. Quantitative trait loci mapping revealed a single locus on chromosome 9 associated with variations in succinate responsiveness. Congenic Balb/c mice carrying the C57BL/6J chromosome 9 locus (Balb.Chr9B6/B6) have elevated baseline numbers of tuft cells and develop tuft cell hyperplasia. The chromosome 9 locus is adjacent to Pou2f3, a transcription factor required for tuft cell differentiation, but does not itself contain any genes associated with tuft cell function. In sum, Balb.Chr9B6/B6 mice demonstrate how baseline tuft cell frequencies determine the threshold of activation for type 2 immune responses in the small intestine and will facilitate discovery of a novel genetic regulator of tuft cell differentiation.

Presenter: Naradikian, Martin

A mesothelin-specific CAR-T cell therapy that incorporates an HLA-gated safety mechanism selectively kills tumor cells

Martin Naradikian, Talar Tokatlian, Grace E Asuelime, Jee-Young Mock, Breanna DiAndreth, Shruti Sharma, Dora Toledo Warshaviak, Mark E Daris, Kristian Bolanos, Breanna L Luna, Kiran Deshmukh, Agnes E Hamburger, Alexander Kamb

A2 Biotherapeutics

Specifically targeting solid tumors while sparing normal tissue continues to vex adoptive cell therapy (ACT) for cancer. An attractive tumor-associated antigen (TAA) expressed in many solid tumors, including lung cancer, is mesothelin (MSLN). However, it is also expressed in vital normal tissues, and investigational MSLN-directed chimeric antigen receptor (CAR) T cells have been toxic. Accordingly, we developed a dual-receptor system (Tmod™) to protect normal tissues while maintaining tumor cytotoxicity in patients with MSLN(+) tumors that have undergone defined loss of heterozygosity (LOH) at the human leukocyte antigen (HLA) locus. Our MSLN Tmod T cells contain a novel MSLN-activated CAR and an HLA-A*02-targeted inhibitory receptor. We demonstrate that MSLN Tmod T cells mediate potent killing of MSLN(+)HLA-A*02(-) tumor cells comparable to a clinically active, but toxic, CAR. However, upon engagement of MSLN(+)HLA-A*02(+) normal cells the blocker dominates and overrides T cell cytotoxicity. Unlike MSLN CAR-T cells, the Tmod system robustly protects surrogate normal cells even in mixed-cell populations in vitro and in vivo xenograft models. Importantly, the MSLN CAR (and other TAA-specific CARs) can also be paired with other HLA class I blockers, supporting expansion to patients beyond HLA-A*02 heterozygotes. In toto, Tmod provides a route to leverage LOH in solid tumors to improve ACT safety.

Presenter: Nelson, Sophia

Progranulin and its role in lymphoid cells during tumor clearance

Sophia Nelson, Nina Serwas, Kelly Kersten, Tristan Courau, Jiasheng Zhang, Megan Ruhland, Kenneth Hu, Eric Huang, Matthew Krummel

UCSF

Progranulin (Grn), a protein mainly expressed in myeloid cells, has been shown to promote migration, invasion, and proliferation in various cancers such as breast, colorectal, and bladder. Interestingly, overexpression of Grn in tumors has also been associated with increased tumor recurrence and worse survival outcomes. However, the exact mechanisms are unknown. Here, we find that Grn-/- mice have a reduced sensitivity to B16 melanomas, with some mice clearing the tumors spontaneously and being unresponsive to tumor rechallenges. Despite Grn being mainly expressed in myeloid cells, this clearance is dependent on the presence of CD4 and CD8 T cells. Neither in-depth flow cytometric analysis nor single-cell RNA sequencing revealed substantial changes in immune cell infiltration. Differential gene expression analysis revealed reduced MHCII and increased cytokine expression in infiltrating myeloid cells in Grn-/- mice, while lymphoid cells were mostly unchanged on the transcriptional level. Metabolically, infiltrating CD8 T cells show increased mitochondrial function and lipid turnover. Isolated CD8 T cells from Grn-/- mice display increased survival, activation, and proliferation ex vivo in comparison to their wild-type (WT) counterparts. In addition, Grn-/- CD8 T cells also exhibit significantly increased production and secretion of pro-inflammatory cytokines IFNγ, IL-2, and TNFα both ex and in vivo. In summary, we show that Grn directly impacts T cell biology, with loss of Grn leading to a stronger CD8 effector response. Future work is needed to determine whether and how absence of Grn could be harnessed in immune-mediated treatments of tumors.

Presenter: Njume, Ferdinand

The GPI anchor and its role in humoral immunity to Toxoplasma gondii

Ferdinand Njume, Jessica N. Wilson, Julia Alvarez, Rachel Szymanski, Kirk D.C. Jensen

UC Merced

Glycosylphosphatidylinositols (GPI) are glycolipid modifications found in all Eukaryotic cells and some archaea such as Eubacteria. In Eukaryotic cells, they have evolved as an arm of anchorage for cell surface proteins, herein referred to as GPI anchored proteins (GPI-APs) where they redirect these proteins preferentially to lipid rafts of cell membrane. Globally, parasites express a higher proportion of GPI-APs on their surface as opposed to non-parasitic eukaryotic cells whose preferred method of anchorage is by use of transmembrane protein domains. In the current study, we sought to investigate the role of the GPI anchor in immunity against parasitic diseases using the apicomplexan model Toxoplasma gondii. Previous research implicates a direct stimulation of TLRs by free GPIs and recognition by early IgM responses. Our results indicate that removal of the diacylglycerol chain from the GPI moiety of GPI-APs using phospholipase C drastically reduces antibody recognition of GPI-APs from T. gondii. To further provide clarity to the role of the GPI anchor in immune recognition, we have employed the use of Me49 T. gondii strains deficient in the GPI-AP SAG1 as well as ME49 strains with SAG1 being anchored by the transmembrane region of CD46. Assessment of antibody response to these strains will be carried out by western blot on T. gondii crude extract panels as well as ectopically expressed SAG1 in HEK cell lysates. To further provide clarity, we have designed SAG1 RNA constructs either lacking the GPI anchor or having CD46 transmembrane anchorage replace the GPI for expression in mice by nano lipoprotein delivery of RNA in order to determine the immune response against the GPI anchor in a qualitative and quantitative fashion. Taken together, these experiments will throw light into the mystery of the role of the GPI anchor in the immune response against parasitic diseases.

Presenter: Nutt, Sam

c-Jun overexpression maintains high surface CAR levels and enhances CAR-T cell activity in an autochthonous model of non-small cell lung cancer

Sam Nutt, Megha Sarvometha, Sarah Garrison, Shivani Srivastava

Fred Hutchinson Cancer Research Center, Human Biology Division

CAR-T cells have been highly successful in treating B cell malignancies, but this success has yet to extend to solid tumors. In a phase 1 clinical trial in patients with breast and lung cancer, CAR-T cells targeting the tumor-associated antigen ROR1 rapidly upregulated multiple inhibitory receptors and lost the ability to produce effector cytokines, adopting a terminally exhausted phenotype. These results indicate that preserving CAR-T cell function will be crucial for their success in solid tumors. T cell exhaustion is characterized by a progressive decline in function in response to chronic antigen stimulation. Imbalance of AP-1 transcription factors is a leading driver of T cell exhaustion, and overexpression (OE) of the activating AP-1 family member c-Jun enhances CAR-T cell function in immune-deficient mouse xenograft models. In addition to chronic antigen stimulation, multiple factors in the suppressive tumor microenvironment (TME) are known to further dysregulate AP-1 balance in T cells. Whether c-Jun OE is sufficient to maintain AP-1 balance and preserve CAR-T cell function within a clinically relevant, immunosuppressive TME is still unknown. To study the impact of c-Jun OE in a more clinically relevant solid tumor model, we adapted the KrasLSL-G12D/+;p53f/f (KP) genetically engineered mouse model of lung adenocarcinoma to express the CAR target ROR1. In the KP-ROR1 model, induced mutations in lung epithelia result in tumors that develop a suppressive TME resembling human disease. While wild-type (WT) CAR-T cells quickly became dysfunctional in the KP-ROR1 model, matching results seen in patients, c-Jun CAR-T cells exhibited enhanced function, resulting in improved tumor control and survival of KP-ROR1 mice. Interestingly, the majority of infiltrating WT CAR-T cells downregulated surface CAR to levels unable to recognize tumor antigen, while c-Jun CAR-T cells maintained high surface CAR levels. As high CAR expression can facilitate greater sensitivity to low antigen expression and enhanced function, we hypothesized that c-Jun OE enhances CAR-T cell function in vivo in part through a novel mechanism of preserving higher surface CAR levels. To evaluate how c-Jun OE alters regulation of surface CAR in a more controlled system, we used plate-bound recombinant ROR1 to stimulate CAR-T cells in vitro. After 24 hours of stimulation, both WT and c-Jun CAR-T cells downmodulated surface CAR levels to the same extent, indicating that c-Jun OE does not prevent CAR downregulation. After 72 hours, however, c-Jun CAR T cells replenished CAR to the surface almost to their original levels while WT CAR-T cells retained low surface CAR levels, indicating that c-Jun OE improves recovery of surface CAR levels after antigen engagement. This rebound in surface CAR expression occurred in c-Jun CARs carrying either CD28 or 4-1BB costimulatory domains. To evaluate whether high CAR expression in c-Jun CAR-T cells is associated with improved function in vivo, we sorted c-Jun CAR-T cells expressing either high (CARhi) or low (CARlo) levels of surface CAR from KP-ROR1 tumors and analyzed transcriptional signatures by RNAseq. Genesets associated with T cell effector function were enriched in c-Jun CARhi T cells, consistent with previous work showing higher surface CAR is associated with enhanced function. c-Jun CARhi cells, however, also expressed higher levels of surface markers associated with exhaustion (PD-1, Tim-3), relative to c-Jun CARlo cells, suggesting that the benefits of maintaining higher surface CAR may concomitantly drive c-Jun CAR-T cell exhaustion. This work illustrates that c-Jun OE positively regulates surface CAR expression, which is a novel mechanism by which c-Jun may improve CAR-T cell performance that may be independent of its role in regulating T cell differentiation. Our findings also suggest that targeting surface CAR regulation is a promising strategy to enhance CAR-T cell function in solid tumors.

Presenter: O'Brien, Valerie

Helicobacter pylori and associated chronic inflammation drive the expansion of a novel, highly proliferative epithelial lineage in a gastric metaplasia model

Valerie O'Brien, Meera K. Shenoy, William C. Young, Greg Finak, Raphael Gottardo, Meghan Koch, Nina R. Salama

Fred Hutchinson Cancer Research Center

At least 80% of gastric cancer cases are attributed to stomach infection with the bacterium Helicobacter pylori (Hp), which causes lifelong chronic inflammation. In some individuals, this inflammation can cause gastric atrophy, metaplasia (conversion of one normal cell type to another), dysplasia (presence of abnormal cells) and finally cancer, but the specific mechanism(s) through which Hp triggers this cascade are not well understood. Similar preneoplastic changes are recapitulated in a transgenic mouse model through tamoxifen-induced expression of active KRAS in the chief cells of the stomach (KRAS+ mice). We found that Hp infection of KRAS+ mice exacerbated disease: compared to Hp-KRAS+ mice, Hp+KRAS+ mice had an altered trajectory of metaplasia and accelerated dysplasia. In accordance with the hypothesis that Hp causes cancer through eliciting chronic inflammation, Hp+KRAS+ mice had severe inflammation marked by a ten-fold increase in gastric lamina propria T cells compared to Hp-KRAS+ mice. To understand gene expression changes in gastric cells in the context of metaplasia with and without active Hp infection, we performed single cell RNA-sequencing (scRNA-seq) on eight mouse stomachs (+/- Hp, +/- KRAS) using the 10x Chromium platform. Cluster analyses followed by UMAP (Uniform Manifold Approximation and Projection) visualization allowed us to identify 25 clusters, including epithelial and immune cell types. Hp+KRAS+ mice had a striking expansion of a population of metaplastic cells (most similar to surface mucus-producing “pit” cells), which comprised ~40% of epithelial cells in these mice. Metaplastic pit cells expressed markers of intestinal metaplasia and gastric cancer, and their abundance in the stomach was correlated with TH1 cell abundance. Analysis of RNA velocity suggested that metaplastic pit cells arose from a progenitor cell type, rather than arising from pit cells directly. Antibiotic eradication of Hp prevented metaplastic pit cell development and other disease phenotypes. Strikingly, oral dexamethasone suppressed gastric inflammation and reduced the abundance of metaplastic pit cells, without impacting Hp loads. Taken together, these studies provide new understanding of how Hp infection and associated inflammation cause gastric preneoplastic progression.

Presenter: Orozco, Susana L.

The role of inflammatory hemophagocytes in malaria-induced anemia and thrombocytopenia

Susana L. Orozco, Holly M. Akilesh, Marion Pepper, Jessica A. Hamerman

Benaroya Research Institute; Department of Immunology, University of Washington

Malaria is a disease caused by protozoan parasites of the Plasmodium species and is a significant global health issue. Disease symptoms range from mild to severe and can result in mortality, especially in the most vulnerable groups – young children and pregnant women. Severe malaria is associated with a variety of complications, including severe malarial anemia (SMA) and thrombocytopenia. The mechanisms leading to SMA are not well understood, although dysregulated immune responses have been implicated. Here we show that specialized phagocytes, termed inflammatory hemophagocytes (iHPCs), developed in a nonlethal murine model of blood-stage malaria. We found that iHPCs differentiated from Ly6Chi monocytes during Plasmodium yoelii 17XNL infection, and numbers of iHPCs correlated with anemia and thrombocytopenia. Although signaling through MyD88 and Unc93b1 was necessary for the development of iHPCs during infection, individual deletion of TLR7 or TLR9 had only modest effects on disease. We have identified iHPCs that have phagocytosed red blood cells and Plasmodium parasites by flow cytometry, and are studying signals and receptors that license iHPCs to consume red blood cells and platelets under these inflammatory conditions. We hope to ultimately provide a better understanding of the mechanisms leading to malarial anemia, which could potentially elucidate intervention strategies or therapeutic targets that could reduce illness and mortality during malarial anemia.

Presenter: Ortiz-Espinosa, Sergio

Complement C5a induces the formation of neutrophil extracellular traps by myeloid-derived suppressor cells to promote metastasis

Sergio Ortiz-Espinosa (1,2,3), Xabier Morales(1,3,4), Yaiza Senent(1,2,3), Diego Alignani(3,5,6), Beatriz Tavira(1,3), Irati Macaya(1),
Borja Ruiz(1), Haritz Moreno(1), Ana Remirez(1,3,6), Cristina Sainz(1,3,6), Alejandro Rodriguez-Pena(1,3,4), Alvaro Oyarbide(1,3,4),
Mikel Ariz(1,3,4), Maria P Andueza(7), Karmele Valencia(1,2,3,6), Alvaro Teijeira(8), Kai Hoehlig(9), Axel Vater(9), Barbara Rolfe(10),
Trent M Woodruff(10), Jose Maria Lopez-Picazo(3,7), Silvestre Vicent(1,3,6,11), Grazyna Kochan(3,12), David Escors(3,12),
Ignacio Gil-Bazo(1,3,6,7), Jose Luis Perez-Gracia(3,6,7), Luis M Montuenga(1,3,6,11), John D Lambris(13), Carlos Ortiz de Solorzano(1,3,4,6), Fernando Lecanda(1,3,6), Daniel Ajona(1,2,3,6), Ruben Pio(1,2,3,6)

(1) Program in Solid Tumors, Cima-University of Navarra, Pamplona, Spain. (2) Department of Biochemistry and Genetics, School of Sciences, University of Navarra, Pamplona, Spain. (3) Navarra's Health Research Institute (IDISNA), Pamplona, Spain. (4) Imaging Platform, CIMA, Pamplona, Spain. (5) Cytometry Unit, Cima-University of Navarra, Pamplona, Spain. (6) Centro de Investigación Biomédica en Red Cáncer (CIBERONC), Madrid, Spain. (7) Department of Oncology, Clínica Universidad de Navarra, Pamplona, Spain. (8) Program in Immunology and Immunotherapy, Cima-University of Navarra, Pamplona, Spain. (9) Aptarion biotech, Berlin, Germany. (10) School of Biomedical Sciences, Faculty of Medicine, University of Queensland, Queensland, Australia. (11) Department of Pathology, Anatomy and Physiology, School of Medicine, University of Navarra, Pamplona, Spain. (12) Immunomodulation Group, Navarrabiomed-Biomedical Research Center, Pamplona, Spain.(13) Department of Pathology and Laboratory Medicine, University of Pennsylvania,

Myeloid-derived suppressor cells (MDSCs) play a major role in cancer progression. We investigated the mechanisms by which complement C5a increases the capacity of polymorphonuclear MDSCs (PMN-MDSCs) to promote tumor growth and metastatic spread. PMN-MDSCs stimulated with C5a favored the invasion of cancer cells via a process dependent on the formation of neutrophil extracellular traps (NETs). NETosis was dependent on the production of high mobility group box 1 (HMGB1) by cancer cells. Moreover, C5a induced the surface expression of the HMGB1 receptors TLR4 and RAGE in PMN-MDSCs. In an in vivo lung metastasis model, inhibition of C5a, C5a receptor-1 (C5aR1), or treatment with NETosis inhibitors reduced the number of circulating-tumor cells, the NETosis in primary tumors and the metastatic burden. In support of the translational relevance of these findings, C5a was also able to stimulate NETosis in PMN-MDSCs obtained from lung cancer patients. Furthermore, myeloperoxidase (MPO)-DNA complexes, as markers of NETosis, were elevated in lung cancer patients and significantly correlated with C5a levels. In conclusion, C5a induces the formation of NETs from PMN-MDSCs in the presence of cancer cells, which may facilitate cancer cell dissemination and metastasis.

Presenter: Othy, Shivashankar

Regulatory T Cells Dance to Protect the CNS: Spatiotemporal Dynamics of Immune Cells in the Spinal Cord During Autoimmune Neuroinflammation

Shivashankar Othy, Amit Jairaman, Joseph L. Dynes, Tobias Dong, Cornelia Tune, Andriy V. Yeromin, Angel Zavala, Ian Parker, and Michael D. Cahalan

University of California Irvine

Regulatory T (Treg) cells not only mitigate autoimmune disorders but also help to resolve tissue inflammation and orchestrate repair pathways, including remyelination in the central nervous system (CNS). However, our understanding of how Treg cells fine-tune local inflammation in target tissues is still incomplete. Using a combination of multiscale two-photon microscopy and our ratiometric calcium reporter, Salsa6f, we uncover new insights into the spatial organization and cellular interactions of Treg cells, Th17 cells, and antigen-presenting cells in the spinal cord at three different phases of experimental autoimmune encephalopathy (EAE): Onset, Peak, and Chronic phases. Th17 cells are primarily localized to the lumbar region at the onset of EAE, increase in numbers and spread along the entire spinal cord at the peak, and then decline in numbers during the chronic phase. Tregs subsequentially infiltrate the spinal cord and co-localize with Th17 cells at the peak and are maintained during the chronic phase. Despite similar speeds, Tregs and Th17 cells display divergent motility patterns during EAE. Th17 cells actively migrate along leptomeninges to spread neuroinflammation across the spinal cord. In contrast, Tregs remain organized as discrete clusters near perivascular APCs. Treg cells within these niches exhibit an unusual repetitive scanning behavior, allowing rapidly motile Tregs to be relatively confined while scanning the APC surfaces. We propose that this unique behavior of Treg cells limits APC:Th17 cell interactions and curtails calcium signals in Th17 cells at a population level. We also prove that Tregs interfere with proximal TCR signaling in Th17 cells, but the final steps of store-operated calcium entry are conserved. We provide evidence for direct collaboration between Treg cells and neural stem cells (NSCs) for promoting remyelination during EAE. Finally, our genetic deletion studies show that mechanically-activated Piezo1 channels selectively restrain the expansion of Treg cells, but not helper T cells. Thus, targeting Piezo1 channels opens new horizons for Treg-based therapies for CNS autoimmunity. In summary, our results demonstrate choreography-based suppression by Treg cells to limit neuroinflammation caused by encephalitogenic T cells.

Presenter: Oviedo, Juan Marcos

IL-4 Signaling Regulates the Fate of B Cell Differentiation and limits BCR repertoire

Juan Marcos Oviedo, Lisa Gibbs, Kimra James, Keke Fairfax

University of Utah

Il-4 has a critical role in organizing the peripheral lymph nodes in homeostasis and after vaccination. To further understand the molecular mechanisms and their impact in the immune response, we used single cell RNA-Seq of CD45+ cells in 4get and 4getIL4R-/- mice following tetanus/diphtheria immunization. We found that the lack of IL-4 signaling restrains the expression of key co-stimulatory and differentiation molecules CD83, CD86, and xbp1, while upregulating the jun/junb/fos pathways. These alterations result in reduced B cell proliferation and differentiation into plasma cells. Surprisingly, we found that in the absence of IL-4 signaling the ratio of dark to light zone residing germinal center shifts from the normal ratio to one dominated by the dark zone. The increase in Dark Zone germinal centers lead to an increase in the diversity of the repertoire in vaccinated 4getIL4R-/- mice as confirmed by VDJ-seq. We also observed that the upregulation of the transcription factor T-bet has the capacity of inducing a similar phenotype. Finally, even though the amount of memory B cells was increased in the absence of IL-4 signaling, we observed that these cells are not able to respond to a secondary challenge. These data suggest a pivotal role for IL-4 in governing B-cell differentiation and the development of optimal cellular and humoral immunity to vaccination.

Presenter: Pandori, William

Single cell immune profiling of convalescent COVID-19 patients identifies long-lasting changes in T cell and monocyte subsets and CD9 expression which correlate with disease severity

William Pandori, Lindsey E. Padgett, Norma A. Gutierrez, Ahmad Alimadadi, Huy Q. Dinh, Serena J. Chee, Claire E. Olingy, Runpei Wu, Daniel J. Araujo, Pandurangan Vijayanand, Christian H. Ottensmeier, and Catherine C. Hedrick

La Jolla Institute for Immunology

Coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 infection is associated with wide immune system dysfunction which can result in severe symptoms, hospitalization and death. While some recover quickly, a substantial population develop long-haul COVID-19 and continue to experience symptoms months after infection. Although significant progress has been made in understanding the adaptive immune response to SARS-CoV-2 during acute infection, gaps still remain in our understanding of how innate immune cells may change across time after SARS-CoV-2 infection. We hypothesized that by using cytometry by time of flight (CyTOF) to analyze PBMCS from healthy and infected subjects, we would identify new cell surface markers and subsets of innate immune cells that change in COVID-19 patients and correlate with clinical parameters associated with COVID-19 severity. In this pursuit, we analyzed PBMCs from 8 healthy, 7 non-hospitalized and 20 hospitalized blood donors and identified monocyte and dendritic cell subsets that changed in frequency during acute SARS-CoV-2 infection and were correlated with clinical parameters of disease severity. The tetraspanin CD9, CD163 and PD-L2 were the only markers that significantly increased in expression in infected subjects. We also found that after approximately 3 months, CD9+ monocytes remained significantly increased and nonclassical monocytes remained significantly decreased in hospitalized subjects' PBMCs. These data identify new monocyte subsets present in the blood of acute COVID-19 patients, and mark CD9, for the first time, as an important cell surface marker that may influence COVID-19 severity. Also, our data suggest that SARS-CoV-2 infection can induce long-lasting changes in the myeloid cell compartment, which could be related to complications observed in long-haul COVID-19.

Presenter: Park, Emily (Minhee)

Understanding the differentiation of conventional monocytes into tumor-supporting metastasis-associated macrophages (MAMs)

Emily (Minhee) Park, Mark Headley

University of Washington (Department of Immunology), Fred Hutchinson Cancer Research Center

Conventional monocytes (cMonos) and macrophages are major accomplices in solid tumors' efforts to metastasize to distal tissues. Our lab recently reported that cMonos get recruited to the lungs in response to metastatic tumor assault in mice and differentiate into populations of tumor-supporting metastasis-associated macrophages (MAMs). cMonos are critical in metastatic tumor establishment as they prime pre-metastatic niches to support survival and colonization of circulating tumor cells. Their progeny, MAMs, have been shown by our lab and others to dramatically enhance metastasis. Despite clear evidence that monocyte-derived macrophages support metastasis, we do not fully understand the factors that drive their differentiation. It is known from in vivo and in vitro studies that upregulation of CSF-1 signaling is indispensable for monocyte-to-macrophage differentiation, but numerous studies have suggested that the signals that cMonos encounter in their surrounding environment, both at steady state and during inflammation, highly influence their differentiation and the functions of their progeny. In alignment with these findings, we observed that cMonos entering the same metastatic niche in murine lungs differentiate into at least two phenotypically distinct populations of MAMs distinguishable by expression of the adhesion receptor, VCAM. Furthermore, key transcriptomic and epigenomic differences between these subpopulations are tied to ingestion of tumor, with the VCAM-hi subpopulation showing features of ingestion. We hypothesize that phagocytosis of tumor material leads cMonos down a distinct differentiation trajectory, resulting in phenotypic and functional diversity in MAMs in the early metastatic niche in the lung. To test our hypothesis, we will collect high-dimensional transcriptomic, epigenomic, and proteomic data to infer cMonos' differentiation trajectories into two MAM subpopulations in metastatic lungs. We will further examine the function of these discrete macrophage subpopulations to better understand their roles in tumor metastasis to the lung.

Presenter: Parmar, Rajesh

Association of Serum IL-10 level and T cell Signaling Genes with Resolving Outcome of MRSA Bacteremia

Rajesh Parmar, Harry Pickering, Richard Ahn, Maura Rossetti, David W. Gjertson, Felicia Ruffin, Vance G. Fowler, Jr., Michael R. Yeaman,
and Elaine F. Reed with the MRSA Systems Immunology Group.

Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA

Persistent Staphylococcus aureus bacteremia (SAB) is prevalent and potentially lethal, particularly when involving methicillin-resistant S. aureus (MRSA). Persistent SAB occurs when the infecting isolate is not cleared from the bloodstream, despite appropriate treatment with an antibiotic to which it exhibits susceptibility in vitro. The host-pathogen relationship resulting in resolving vs. persistent SAB is poorly understood. Thus, further studies are necessary to understand persistence or resolution of SAB due to MRSA. Here, we investigated transcriptional signatures associated with persistent vs. resolving MRSA SAB. We assessed serum IL-10 cytokine levels by Luminex and whole-blood transcriptome of patients with MRSA infection by RNA-seq. Persistent SAB was defined as blood culture-positive within five days after vancomycin treatment. Thirty patients resolved their infection and 28 had persistent infection. Co-expressed genes were combined into gene expression (GE) modules by weighted gene co-expression network analysis. The GE pathways enriched in each module were identified using Reactome pathway analysis and protein-protein interactions (PPI) network via STRING Interactome. Signatures of persistent vs. resolving SAB were identified by integrating identified GE modules with plasma IL-10 cytokine. We identified 58 GE modules. Expression of three modules was increased in patients with resolving SAB infection. Pathway analysis of these modules demonstrated enrichment in pathways related to T cell signaling. Of these, the most highly connected genes were involved in T cell signaling pathways correlating with low plasma IL-10 levels. The present findings show that persistence vs. resolution of MRSA SAB involves dysregulation of host adaptive immune responses. Understanding transcriptional profiles associated with clinical outcomes relative to cytokine production may lead to improved understanding of S. aureus pathogenesis and identification of prognostic factors for interventions to improve outcomes in MRSA infection.

Presenter: Peng, Changwei

Engagement of ICOS in tissues promotes establishment of CD8+ tissue-resident memory T cells

Changwei Peng, Matthew A. Huggins, Kelsey M. Wanhainen, Todd P. Knutson, Hanbin Lu, Hristo Georgiev, Kristen L. Mittelsteadt, Nicholas N. Jarjour, Haiguang Wang, Kristin A. Hogquist, Daniel J. Campbell, Henrique Borges da Silva, Stephen C. Jameson

University of Minnesota

Recirculating and tissue-resident memory CD8+ T cells provide distinct modes of immune protection, yet the signals that dictate differentiation of these populations are ill-defined. In particular, the interactions within tissues that promote generation of resident memory T cells (TRM) are unclear. Here, we show that the inducible costimulatory molecule ICOS, well known to regulate differentiation of CD4+ T cell populations, is required for CD8+ TRM but not recirculating memory subsets. Furthermore, ICOS engagement during CD8+ T cell recruitment to non-lymphoid tissues is critical for efficient TRM establishment: ICOS/ICOS-L interactions are dispensable throughout CD8+ T cell priming and for TRM maintenance, while ICOS-L expression by radioresistant cells is key for optimal TRM generation. This role for ICOS depends on its ability to signal through PI3K. Together, our data illustrate that specific local costimulatory cues promote production of tissue-resident populations, with potential implications for therapeutic manipulation.

Presenter: Posada, Jasmine

Toxoplasma Gondii: Host Death Mediated through GPI-anchor Modification?

Jasmine Posada, Julia Alvarez, Ferdinand N. Njume, Scott P. Souza, Juan C. Sanchez-Arcila, Kirk DC Jensen

University of California, Merced

The obligate intracellular parasite *Toxoplasma gondii* is a leading cause of death attributed to foodborne illness in the United States. In endemic areas, more than 60% of some populations can be infected with *T.gondii*. In order to aid in eliminating this globally spread parasite our focus is to further understand how this organism achieves a productive infection. The surface of *T.gondii*. is known to be enriched with various glycolipids that allow for motility of the parasite, cellular adherence and host cell invasion. Specifically, the glycosylphosphatidylinositol (GPI)-lipid anchor is a highly conserved glycolipid that anchors proteins to the external membrane of *T.gondii*. and are known to be highly immunogenic and trigger a robust IgM antibody response. With the use of CRISPR Cas9 technology, we have identified and removed the parasite's glycosyl transferase responsible for adding branching sugars to the mannose core of the *T.gondii*. GPI anchor. The mutant parasites completely lack the GPI side chain, and its GPI antigen is no longer recognized by IgM after infection. These GPI side chain mutants also have increased virulence resulting in increased host death after primary infection. In an effort to understand the mechanism of this increase in virulence, we are characterizing various immunological responses and parasite interactions with its host. To connect GPI side-chain modification to host death we are investigating innate cytokine responses, TLR recognition, host cell invasion, parasite dissemination, and brain cyst formation, and we hypothesize to observe significant differences between wild-type and knock-out parasite strains in one or some of these phenotypes.

Presenter: Pruner, Kurt B.

SARS-CoV-2 Hybrid Immunity Drives a Distinct T Cell Landscape from Infection or mRNA Vaccine Alone

Kurt B. Pruner, Lauren B. Rodda, Peter A. Morowski, Christian Howard, Mitchell L. Fahning, Jason Netland, Caleb Stokes, Michael Gale Jr., Daniel J. Campbell, Marion Pepper

Department of Immunology, University of Washington School of Medicine, Seattle, WA 98109, USA

The SARS-CoV-2 pandemic has caused millions of deaths globally and has dramatically restricted societal and economic norms since March 2020. Vaccination with SARS-CoV-2 mRNA vaccines has provided durable and potent protection from severe COVID-19. Interestingly, it has been observed that individuals who have been exposed to both SARS-CoV-2 infection and mRNA vaccination are endowed with superior protection during reinfection events. Although it has been postulated that this phenomenon is due to increased antibody breadth in previously exposed people, it is not fully understood the immune mechanisms at play, and SARS-CoV-2-specific T Cell functional output has not been investigated. To address this, we generated a focused ex vivo cytokine release assay to interrogate the quantity and functional quality of SARS-CoV-2 Spike- and M/N-specific T Cells in Naïve and Previously Infected individuals before vaccination, 2 and 12 weeks after the second dose of mRNA vaccine, and 2 weeks after the third dose (booster) of mRNA vaccine. We observed that Spike-specific CD4 T Cells are induced by both infection and vaccination and are present at similar numbers in Naïve and Previously Infected cohorts after two doses of mRNA vaccine. Further we see no augmentation of Spike-specific T Cells following the booster vaccine dose. M/N-specific T Cells are present in Previously Infected but not Naïve cohorts, persisting for >1-year post-infection. SARS-CoV-2 infection drove production of the cytokines IFN-g, IL-10, and IL-2 in both Spike- and M/N-specific memory CD4 T Cells, whereas vaccination in Naïve cohorts induced a more multipotent response in which IL-4 and IL-21 were also produced by Spike-specific T Cells. Strikingly, IFN-g and IL-10 producing T Cells were present at nearly two-fold levels in Previously Infected individuals relative to Naïve donors after a two-dose mRNA vaccine regimen. Levels of cytokine producers remained stable following third dose of vaccine (booster) in both Previously Infected and Naïve cohorts, suggesting this phenomenon is driven by differential programs elicited by infection or vaccination and not by the number of antigen exposures. Together, these data suggest that individuals with “hybrid immunity” are endowed with a divergent immune landscape to immunization or infection alone and that this phenomenon is not due to the number of antigen exposures, but the environment in which antigen was first perceived.

Presenter: Radu, Caius

Pleiotropic, multigenic-dependent, and immune lineage-specific consequences of restricting the breakdown of guanine nucleosides (Part 1)

Caius Radu, Evan R. Abt, Khalid Rashid, Thuc M. Le, Suwen Li, Hailey R. Lee, Vincent Lok, Alex Lam, Amanda L. Creech, Arthur Cho,
Valerie Rezek, Gabriel Abril-Rodriguez, Liu Wei, Steven Mittelman, Antoni Ribas, Shanta Bantia, Thomas Mehrling, Gay M. Crooks,
Timothy R. Donahue, Ting-Ting Wu

University of California Los Angeles

Purine nucleoside phosphorylase (PNP) functions as a central regulator of nucleotide metabolism by catalyzing the degradation of purine nucleosides guanosine and deoxyguanosine. PNP inactivation has been paradoxically associated with both immunodeficiency and autoimmunity in humans but the genetic and molecular determinants of these divergent outcomes are poorly defined and whether they reflect cell type-specific mechanisms is currently unknown. Moreover, the mechanisms underlying the clinical efficacy of PNP inhibitors in a subset of patients with hematological malignancies are yet to be determined, thereby drastically limiting the potential of this promising new class of metabolic targeted therapies. To address these gaps in knowledge we performed a systematic evaluation of the metabolic, immunological, and cell-context phenotypic effects of PNP inactivation. We show that during T cell development and in malignant cells, PNP loss of function is synthetically lethal with sterile alpha motif and HD domain-containing protein 1 (SAMHD1) deficiency by triggering massive dGTP pool expansion and impairing DNA replication. These cytotoxic effects require the expression of deoxycytidine kinase (dCK), which mediates intracellular trapping of deoxyguanosine and dGTP pool expansion and are opposed by environmental deoxycytidine produced by stromal cells in a SAMHD1 and nucleoside transporter dependent manner. This resistance mechanism to PNP inactivation is counteracted by the deoxycytidine catabolizing enzyme cytidine deaminase (CDA). These findings identify novel multigenic and multicellular circuits that regulate guanosine metabolism, which can be leveraged for the development of novel therapeutic applications.

Presenter: Radu, Caius

Pleiotropic, multigenic-dependent, and immune lineage-specific consequences of restricting the breakdown of guanine nucleosides (Part 2)

Caius Radu, Evan R. Abt, Khalid Rashid, Thuc M. Le, Suwen Li, Hailey R. Lee, Vincent Lok, Alex Lam, Amanda L. Creech, Arthur Cho,
Valerie Rezek, Gabriel Abril-Rodriguez, Liu Wei, Steven Mittelman, Antoni Ribas, Shanta Bantia, Thomas Mehrling, Gay M. Crooks,
Timothy R. Donahue, Ting-Ting Wu

University of California Los Angeles

Purine nucleoside phosphorylase (PNP) functions as a central regulator of nucleotide metabolism by catalyzing the degradation of purine nucleosides guanosine and deoxyguanosine. PNP inactivation has been paradoxically associated with both immunodeficiency and autoimmunity in humans but the genetic and molecular determinants of these divergent outcomes are poorly defined and whether they reflect cell type-specific mechanisms is currently unknown. Moreover, the mechanisms underlying the clinical efficacy of PNP inhibitors in a subset of patients with hematological malignancies are yet to be determined, thereby drastically limiting the potential of this promising new class of metabolic targeted therapies. To address these gaps in knowledge we performed a systematic evaluation of the metabolic, immunological, and cell-context phenotypic effects of PNP inactivation. We show that in addition to, and independent of its dCK and CDA-dependent cytotoxic effects in SAMHD1 deficient cells, PNP inactivation elevates serum inflammatory cytokines and cytokine transcript levels in secondary lymphoid organs. Using scRNAseq we identify CD19+ B-cells and CSF1R+ macrophages, which express the pattern recognition receptor TLR7, as the drivers of these alterations. TLR7 activation in these cell types is promoted by guanosine accumulation which functions as a ligand for TLR7 alongside uridine-containing ssRNA. Importantly, TLR7 stimulation achieved by PNP inhibition initiates transcriptional alterations that are quantitatively and qualitatively distinct from those induced by synthetic TLR7 agonists. We additionally show that PNP inactivation stimulates germinal center responses in secondary lymphoid organs via the expansion of activated B-cells and T follicular helper cells. In summary, we demonstrate that by modulating multiple metabolic and immune checkpoints in a multigenic, cell type, and environmental dependent manner, pharmacological PNP inhibition elicits specific outcomes, which can be leveraged for the development of novel therapeutic applications.

Presenter: Ray, Arja

Defining tumor-reactive CD8 T cell states using a novel T cell activation reporter

Arja Ray, Kenneth Hu, Grace Hernandez, Bushra Samad, Alexis Combes, Matthew Krummel

UCSF

Although chronic activation is considered a major driver of T cell dysfunction in the tumor microenvironment (TME), there is a virtual absence of methodologies to discern how T cells spatiotemporally progress through activation states into that dysfunction. We generated a novel T cell activation reporter mouse, which is a transcriptional reporter of CD69 expressing the fluorescent protein TFP. By recording transcriptional activity of a locus where the protein expression is under tight, activation-dependent post-transcriptional control, we generated a reporting system to read out contemporary as well as historic T cell triggering. While cell surface CD69 reliably indicates current activation, resting TFP (Cd69 mRNA) expression is downregulated with successive TCR triggering and hence an inverse marker of chronic stimulation. Using multiple tumor models in vivo, tumor slice cultures ex vivo and scRNA-Seq, we thus defined and tracked over time distinct functional states of intratumoral T cells based on their activation histories. These data revealed key insights including the delineation of quiescent versus activated states within progenitor, effector and exhausted T cells and the trajectory of inter-conversion that leads to the emergence of these states in the TME. Probing the inflection points in this differentiation trajectory reveals a particularly interesting subset of CD69+TFP+ cells, representing intratumorally activated effector CD8 T cells prior to their dysfunctional adaptation to chronic stimulation. Dissecting their phenotype led to the discovery of a previously unexplored subset of intratumoral T cells of functional significance. Indeed, these cells were enriched specifically in the context of T cell-mediated tumor control in mice and their phenotypic markers correlated with improved immunotherapy outcomes and survival in human cancer patients.

Presenter: Ray, John

Identification of autoimmune disease-associated non-coding genetic variants that perturb enhancer activity in T cells.

John Ray, Kousuke Mouri, Michael H. Guo, Carl G. de Boer, Michelle M. Lissner, Ingrid A. Harten, Gregory A. Newby, Hannah DeBerg, Winona F. Platt, Matteo Gentili, David R. Liu, Daniel J. Campbell, Nir Hacohen, Ryan Tewhey

Benaroya Research Institute

Genome-wide association studies have uncovered hundreds of autoimmune disease-associated loci; however, defining the causal genetic variant(s), most of which are in non-coding regions, and determining their effects in disease-relevant cell types remains a substantial challenge. Here, we test >18,000 autoimmune disease-associated non-coding variants for five autoimmune diseases for variant-modulated cis-regulatory activity in massively parallel reporter assays. We identify 60 putative disease-causal variants that likely play a role in altering T cell function. For one highly conserved variant, we used base editing to confirm a reduction in expression of the target gene BACH2 in a human T cell line, and we introduced a small deletion of the orthologous non-coding sequence in mice, resulting in reduced BACH2 expression in naïve CD8 T cells. T cells from these mice also had reduced expression of genes that suppress activation and maintain T cell stemness, a key function of BACH2, and, upon acute viral infection, variant-deleted naïve T cells are more likely to differentiate into effector cells. Our results represent an example of defining disease-causal variants and studying their immunologically relevant effects, allowing for discovery of disease-risk mechanisms.

Presenter: Rodriguez, Donald

Impact of TCR-pMHCII affinity on the pathogenicity and regulation of prostate-specific CD4+ T cells

Donald M. Rodriguez, Ryan Duncombe, Sharon Zeng, Victoria Lee, Erin J. Adams, and Peter A. Savage
The University of Chicago

In both humans and mice, self-reactive CD4+ T cells are implicated in a range of autoinflammatory processes. To promote disease, such cells must evade clonal deletion in the thymus, as well as intrinsic and extrinsic regulation mechanisms in the periphery, including anergy and regulatory T cell-mediated suppression. However, the impact of T cell receptor (TCR) - peptide/MHC-II binding properties on the pathogenic potential of self-specific cells and their susceptibility to immune regulation remains incompletely defined. Much previous work on this topic has relied on the study of T cells reactive to foreign model antigens, yet immune responses towards such antigens may not recapitulate those targeting bona fide self-antigens. Through the in vivo analysis of CD4+ T cell clones reactive to a natural I-Ab-restricted self-peptide derived from the prostate-specific protein Tcaf3 (termed "C4" peptide), we aim to define the role of TCR - peptide/MHC-II binding affinity on the pathogenicity and regulation of self-reactive CD4+ T cell clones. In both tumor-free and prostate-tumor-bearing mice, our findings thus far reveal notable differences in the developmental trajectories and peripheral functions of clones with varying TCR affinity for the C4/I-A^b self-ligand. Ongoing work will continue to leverage our novel and physiologically relevant TCR-ligand system to better understand the fundamental mechanisms by which immune tolerance is established and enforced.

Presenter: Roncaioli, Justin

Cell death pathways in intestinal epithelial cells define mouse resistance to oral Shigella flexneri challenge

Roncaioli Justin, Mitchell PS, Babirye JP, Liu F, Turcotte EA, Rauch I, Chavez RA, Lee AY, Bergen I, Lesser, CE, Vance RE

UC Berkeley

Shigella flexneri is a human-specific bacterial pathogen that invades and disseminates within the colonic epithelium, causing a severe diarrheal disease that claims >200,000 lives each year. A challenge in studying and combating Shigella-induced disease is the lack of genetically tractable small-animal models that accurately recapitulate human infection and disease. In particular, mice are highly resistant to shigellosis. We hypothesized that protection might be mediated by cell death pathways in intestinal epithelial cells. We have found that mice pre-treated with oral streptomycin and deficient for the NAIP–NLRC4 inflammasome are acutely susceptible to oral Shigella challenge and display key hallmarks of human shigellosis, including robust colonization of intestinal epithelial cells (IECs) and neutrophilic gut inflammation. Mice that express NAIP-NLRC4 only in the intestinal epithelium phenocopy wild-type mice, suggesting that inflammasome expression in IECs alone is sufficient to protect against epithelial cell invasion, spread, and resulting disease. NLRC4-knockout mice additionally deficient for non-canonical inflammasome CASPASE-11 or treated with a TNFα blocking antibody exhibit significant increases in IEC colonization and disease, suggesting that CASPASE-11-dependent pyroptosis and TNFα-dependent apoptosis act in concert with NAIP-NLRC4 to limit bacterial spread in IECs. Our findings provide the first oral infection mouse model of shigellosis and establish key roles for multiple intestinal epithelial cell death pathways in defense against a deadly cytosolic enteric pathogen.

Presenter: Rostomily, Clifford

A systems approach reveals hallmarks of peripheral and tissue immunity during Lyme disease

Clifford Rostomily, Henry Hampton, Akhade Akhade, Mary Brunkow, Leroy Hood, Gary Wormser, Naeha Subramanian

Institute for Systems Biology

Lyme Disease (LD) is an emerging tick-borne zootonic infection caused by the bacterium Borrelia burdorferi that has seen increased incidence and geographic distribution worldwide, with an estimated 300,000 cases in the United States each year. The disease manifests as a heterogeneous array of symptoms of unknown origin that in some individuals do not subside post antibiotic treatment. With the goal of understanding how the immune response contributes to patient symptomology, we recruited a longitudinal prospective cohort of Lyme Disease patients and conducted multiplexed measurements of circulating immune cells and mediators in their blood at four time points up to one year, and compared this data with publicly available transcriptomic data from the site of tick bite (i.e. the skin). We demonstrate that concentrations of many circulating cytokines and chemokines are elevated in patient plasma during acute disease, and are rapidly cleared after a standard course of antibiotics. Lasso regression models trained on these data could classify patients from controls at diagnosis with an AUC = 0.683 after monte carlo cross validation suggesting that the acute circulating protein signature has moderate predictive capability. Analysis of publicly available skin transcriptomic data mapped the circulating cytokine signature to robust immune cell infiltration at the site of tick bite. However, despite a strong circulating protein signature originating from the site of infection, patients exhibited only mild and heterogeneous changes in circulating immune cells even when they had multiple foci of infection on the skin, with only one patient with severe disseminated disease showing dramatic changes in peripheral immune cell abundance and activation. The muted peripheral cellular response was confirmed by cellular deconvolution of publicly available blood transcriptomic studies. Our data reveal a surprising silencing of peripheral acute immune activation in Lyme Disease despite a robust response at the site of infection. These findings also suggest that peripheral immune cell activation is a hallmark only of severe disseminated disease and have implications for understanding how residual or incipient infection in some individuals may escape peripheral immunity to manifest long-term disease sequelae.

Presenter: Roy, Koushik

Role of NFkB in B cell differentiation

Koushik Roy, Simon Mitchell, Alexander Hoffmann

Department of Pathology, University of Utah; Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles

Humoral immunity depends on the efficient activation of B-cells and their subsequent differentiation to antibody-secreting cells (ASCs). How a B cell decides its fate is a long-standing and enigmatic question in immunology. Utilizing single-cell lineage tracing, we revealed deterministic B cell fate decisions as opposed to previously described stochastic B cell fate decisions. The transcription factor NFkB cRel is critical for B-cell proliferation. Here, we found that cRel is dynamically repressed during ASC differentiation, and cRel reduction is required for Blimp1 elevation. Conversely, Blimp1 represses cRel. Including this bi-stable circuit of mutual cRel-Blimp1 antagonism into a multi-scale mathematical model revealed that dynamic control of cRel phases B-cells from proliferation to differentiation into ASCs. Our studies provide a mechanistic explanation of how dysregulation of cRel and Blimp1 results in different B cell lymphoma.

Presenter: Sanchez-Arcila, Juan Camilo

The use of Forward genetic screens and the Collaborative Cross to study adaptive immunity to Toxoplasma gondii

Juan Camilo Sanchez-Arcila, Arlon Wizzard, Jennifer Eggleston, Darian Galvez, Scott P Souza, Kirk DC Jensen

University of California Merced

Toxoplasma gondii, the causative agent of human toxoplasmosis, is distributed worldwide and infects billions of people. Similar to other parasitic infections, immunological responses induced by T. gondii are not sterilizing, leading to the possibility of multiple re-infections during one's lifetime. Additionally, there are no fully protective vaccines for any human parasitic pathogen. In this project, we employ an unbiased forward genetics screen to find novel immunological responses required to protect against a highly virulent strain of T. gondii. The Collaborative Cross (CC) is a panel of multi-parental recombinant inbred mouse lines derived from eight founders of laboratory and wild-derived origin that span the three major Mus musculus subspecies (M. domesticus, M. musculus, M. castaneus). The CC panel captures ~90% of the genetic variation within the Mus musculus species, affording the discovery of novel biological mechanisms. To determine new requirements for immunity to T. gondii, we screened the available panel of 59 CC lines for immunity QTLs generated by vaccination or natural infection with a low-virulent strain. Our model consists of challenging mice with a highly virulent French Guyana strain, GUY-DOS, capable of evading immunological memory responses generated in some but not all founder CC lines. We found a single highly significant Quantitative Trait Locus (QTL) located in a small region on Chromosome (Chr)11 that correlated with survival to GUY-DOS secondary infections in vaccinated or naturally infected animals. The Chr11 QTL accounts for 70% of the total phenotypic variance observed within the panel, and candidates within this region offer clues as to how to promote vaccine-induced immunity to parasites. A variety of immune parameters were also scored, genetically mapped, and will be presented. A further evaluation revealed enhanced memory CD8 T cell, humoral and Tfh responses following vaccination in genetically resistant mice. Thus, genetic variants of the candidates located in Chr11 QTL may explain the immunological basis for enhanced vaccine efficacy observed in mice with a resistant phenotype to a virulent challenge. The results obtained demonstrate the power of forward genetics screens and the use of highly diverse mice panels to study complex immunological traits, such as Toxoplasma secondary infections.

Presenter: Scharping, Nicole

Targeting T cell fates: converting exhaustion to memory to improve immunotherapeutic responses to cancer

Nicole Scharping, Allison Cafferata, Maximilian Heeg, Quynhanh Nguyen, Ananda Goldrath
University of California San Diego

In cancer, CD8+ T cells have the power to target and kill tumor cells with precision, but often fail due to chronic activation from the immunosuppressive tumor microenvironment (TME). T cells that experience prolonged activation in the TME differentiate into a severely dysfunctional cell state known as exhaustion. In healthy tissues, T cells differentiate into tissue-resident memory cells (TRM) in response to infection, which remain lodged in tissues to provide protection from reinfection. When TRM-like cells are found in patient tumors, they are correlated with improved outcomes and responses to immunotherapy. Understanding how to manipulate T cell fates in an effort to prevent exhaustion and favor TRM-characteristics could benefit cancer immunotherapy. To explore differences between these cell states, we screened the core TRM gene-expression signatures for genes downregulated as T cells undergo terminal exhaustion. Targets were then overexpressed in antigen-specific T cells and adoptively transferred into tumor-bearing mice for analysis. Interestingly, many genes related to protein regulation and processing were identified, including a novel gene called Neuralized E3 Ubiquitin Protein Ligase 3 (Neurl3). Neurl3's function is not well described, however, experimentally mutating the RING domain suggests Neurl3 transfers ubiquitin to target proteins for degradation. When Neurl3 was overexpressed in tumor-specific T cells, we found tumor infiltrating lymphocytes still upregulated inhibitory receptors PD1 and Tim3, but showed enhanced anti-tumor function. Neurl3-overexpressing T cells had increased accumulation in the TME, upregulated canonical TRM markers CD69 and CD103, produced more cytokines, controlled tumor growth, and increased mouse survival in B16 melanoma and MC38 adenocarcinoma. These results highlight the understudied field of negative regulation of T cell function by protein degradation in T cell differentiation fate and uncover a potential gene target for immunocellular therapies to favor functional T cell fates in cancer.

Presenter: Shamskhou, Elya

Defining the cellular events of CD4 T cell priming during Mtb Infection

Elya Shamskhou, Jasmine Wilson, Courtney Plumlee, Benjamin Gern, Kevin Urdahl, Michael Gerner

University of Washington, Department of Immunology; Seattle Children's Research Institute

CD4 T cells are required for protective immunity against Mycobacterium tuberculosis (Mtb), the bacterium that causes tuberculosis, but the CD4 T cell response to Mtb infection is heterogenous and not all responses are helpful. To better understand how adaptive response heterogeneity against Mtb is established, we utilized the mouse model of Mtb infection and Mtb-specific TCR transgenic CD4 T cells to investigate the early initiation of T cell priming by antigen presenting cells in lung-draining mediastinal lymph nodes (mLNs). We found that transgenic and endogenous CD4 T cells underwent rapid differentiation into several subtypes of early effector T cells, as characterized by distinct transcription factors. Moreover, multiplexed imaging analysis revealed large aggregates of Mtb-infected myeloid cells in the mLN with decreases in conventional dendritic cell populations, suggesting re-structuring of the mLN architecture in response to Mtb infection. Interestingly, co-localization of Mtb-rich myeloid clusters with Mtb-specific CD4 T cells was observed at early timepoints, while Mtb-specific CD4 T cells were less associated with these Mtb-rich myeloid clusters just a few days later. Future studies will characterize distinct innate microenvironments in lymph nodes and their impact on antigen presentation and generation of CD4 T cell response heterogeneity.

Presenter: Shehata, Laila

Examining the role of IL-4 signaling in the B cell response to infection

Laila Shehata, Marion Pepper
University of Washington

B cells play a significant role in adaptive immunity by differentiating into antibody-secreting cells, whose antibodies can bind to invading pathogens with high affinity and specificity to inhibit infection. This protective response relies on germinal centers (GCs), specialized structures that form following pathogen exposure and allow for affinity maturation and selection of class-switched memory B cells (MBCs) and long-lived plasma cells (LLPCs). T follicular helper (Tfh) cells are critical for a proper GC response, as they direct B cell selection and differentiation through both direct receptor-ligand interactions and the cytokines IL-4 and IL-21. The role of Tfh-derived IL-4 on B cells is not well understood, yet has been suggested to promote GC formation by promoting the expression of BCL6, a transcription factor that regulates the GC through its roles as a transcriptional repressor and anti-apoptotic signal. B cells are required to upregulate BCL6 to form and enter GCs, but must downregulate it to exit as MBCs or LLPCs. Surprisingly, when we administered IL-4 to Plasmodium-infected mice, there was a significant reduction in antigen-specific B cells, potentially due to an observed drop in BCL6 expression in both GC B cells and the transitory CD38+GL7+ B cell population. In vitro cultures of B cells with or without IL-4 showed that IL-4 upregulates BCL6 expression on naïve B cells, but downregulates it on GC B cells. Together, this suggests that IL-4 may play a dual role in regulating B cells and their entry into and exit from the germinal center, which may impact memory B cell formation.

Presenter: Shenoy, Meera K

Breastmilk IgG imprints host-microbiota mutualism in neonates

Meera K Shenoy, Diane M Rico, Meghan A Koch

Fred Hutchinson Cancer Center

To establish and maintain intestinal homeostasis, the immune system must mount sterilizing inflammatory responses to pathogens yet remain tolerant to beneficial or innocuous commensal microbiota. This process is particularly complicated in early life, when the neonatal immune system encounters new and changing antigens due to, for example, shifts in diet. As such, defining the mechanisms that regulate host-microbiota interactions in early life is an important area of research. Using a mouse model of maternal antibody deficiency, we demonstrated that ingestion of maternal IgG in the first week of life restrains microbiota-dependent adaptive immune responses during the weaning transition (3-4 weeks of age) via CCR7-dependent cell migration. Compared to neonates fed IgG in the first week of life, neonates that only receive maternal IgG in utero or after the first week of life mount heightened gut-associated T follicular helper cell (Tfh) and germinal center (GC) B cell responses following weaning. This dysregulated weaning reaction is not driven by maternal IgG-mediated alterations in gut microbiota composition or intestinal barrier function. Interestingly, administration of broad-spectrum antibiotics in the first week of life led to similar increases in Tfh and GC B cell responses during the weaning transition, suggesting that maternal IgG binding to cognate microbial antigen is required to restrain neonatal microbiota-reactive adaptive immunity. Here we identify a novel mechanism by which maternal IgG regulates nascent microbiota-reactive adaptive immunity. These exciting findings elucidate mechanisms by which maternal microbiota-reactive IgG directly imprints the neonatal intestinal immune system and shed light on how breastfeeding can have long-lasting consequences for host-microbiota mutualism.

Presenter: Shixin, Ma

Metabolic control of epigenetic states that drive CD8 T cell exhaustion and anti-tumor immunity

Ma Shixin, Michael Dahabieh, Won-Suk, Steven Zhao,Tom Mann,Bryan McDonald

salk

The presence of tumor infiltrating lymphocytes (TILs) in tumors represents a positive prognostic marker for clinical success. Cytotoxic CD8 TILs are particularly relevant cells for recognizing and destroying cancer cells. However, as tumors progress, the CD8 TILs often become immunosuppressed or functionally exhausted (i.e., dysfunctional). This TIL dysfunction permits tumors to persist and grow and likely occurs in response to prolonged antigenic stimulation and other inhibitory signals or cell types in the tumor microenvironment (TME) such as PD-1, CTLA-4 and regulatory T cells (Tregs). Immunotherapies such as anti-CTLA-4 and anti-PD-1 have proven remarkably efficacious in enhancing anti-tumor T cell responses in some cancer patients, indicating that drugs that target TIL exhaustion present promising clinical opportunities. Unfortunately, still only a minority of patients have complete and durable responses to these drugs. Therefore, while the pathways and processes that drive CD8 T cell exhaustion are becoming clearer, it is urgent that we discover new methods to interfere or reverse this process from occurring in tumors. We showed that TEX cells have reduced nuclear levels of ACSS2, which synthesizes acetyl-CoA from acetate, along with decreased nuclear acetyl-CoA levels, histone acetylation and cytokine production. As a result, the decrease of ACSS2 in CD8 T cells leads to impaired anti-tumor immune responses.

Presenter: Stanbery, Alison

Memory Th2s integrate into the tuft-ILC2 circuit to provide protective immunity to helminth infection

Alison Stanbery, Lily Webeck, Jakob von Moltke

University of Washington

Both helminth infection and allergic responses involve type 2 immune activation at barrier tissues. Type 2 immune activation is characterized by cell recruitment, mucus production, and tissue remodeling mediated by IL-13-secreting ILC2s and T helper 2 (Th2) cells. When activated chronically by allergens, tissue-resident Th2 cells (Th2 Trm) can cause morbidity, while ILC2s and circulating Th2s play important roles in clearing helminth infection. During acute helminth infection in the small intestine, epithelial tuft cells secrete IL-25 to activate ILC2s. ILC2-derived IL-13 acts on epithelial crypt progenitors to promote differentiation and increased frequency of tuft cells, thereby establishing a feed-forward tuft-ILC2 circuit that mediates epithelial remodeling and worm expulsion. It is unknown if Th2 cells can integrate into this circuit. Here we show that tuft cells contribute to Th2 Trm generation, and that Th2 Trm regulate tuft cell frequency. Using an in vivo model to permanently label and track cytokine-producing Th2 cells, we found distinct populations of lineage-traced Th2 Trm within intestinal and peripheral tissues. In particular, intestinal Th2 Trm express the receptor for IL-25, while Th2 Trm in adipose tissues express the IL-33 receptor. Loss of tuft cells results in a defect in the generation of Th2 Trm, leading to greater worm burdens and a defect in serum levels of IgE following reinfection. Finally, we found that helminth-induced Th2 are necessary for tuft cell hyperplasia during chronic primary infection and sufficient to induce tuft cell expansion and worm clearance during reinfection. Together, our data indicate that the tuft-ILC2 circuit can be rewired to incorporate Th2 cells and provide the first evidence that intestinal tuft cells contribute to adaptive immunity to helminths.

Presenter: Stolley, Michael

Leveraging Resident Memory T Cells to Fortify Oral Immunity

Michael Stolley, David Masopust

University of Minnesota

Resident memory T cells (TRM) durably survey barrier tissues for reinfection and orchestrate collaborative immune responses upon activation. Importantly, peptides derived from previously encountered viral infections can locally reactivate TRM. This biology may have convenient therapeutic ramifications for augmenting tissue-specific immunity. Yet, while TRM have been well characterized in the gut, skin, and urogenital mucosa, the mouth remains a barrier tissue largely unexplored by T cell biologists. We asked whether TRM in the oral mucosa could be reactivated using virus-mimicking peptides, and if so, how that might impact oral immunity. This question was addressed using a novel prime-pull model for generating preternaturally abundant TRM in the mouths of SPF mice to manipulate and study. Oral TRM reactivation perpetuated a robust oral anti-pathogen state, including induction of key antiviral and interferon-stimulated genes, and recruitment of innate and adaptive cells into the oral mucosa. Oral peptide pre-exposure thwarted infection with an antigenically unrelated virus. Thus, mouth-resident T cells are amenable to local peptide reactivation, and their proinflammatory potential can be intentionally deployed to bolster oral immunity.

Presenter: Tabilas, Cybelle

Early microbial exposure shapes immunity by altering CD8+ T cell ontogeny

Cybelle Tabilas, David Iu, CCiarán W.P. Daly, Samantha P. Wesnak, Norah L. Smith, Miles P. Davenport, Andrew W. Grimson, and Brian D. Rudd

Cornell University

Microbial exposure during critical stages of development can have long-lasting effects on the health of an individual. However, the mechanisms of how microbial exposure in early life leads to permanent changes in the immune system have not been elucidated. In this study, we compared immune development and function in laboratory mice raised in either a 'clean' (SPF) or after exposure to 'dirty' environment (pet-shop). We found adult mice raised in a dirty environment are highly resistant to infection because of changes to the developmental architecture of their CD8+ T cell compartment. Exposure to a dirty environment led to increased survival of fast-acting fetal-derived CD8+ T cells and the adoption of a more effector-like epigenetic landscape. We also performed thymic transplant experiments, which indicated microbial programming occurs during early stages of T cell development. Given that development in a dirty environment led to an expansion of highly reactive fetal-derived cells, we next asked whether removing the fetal-layer in dirty mice would increase susceptibility to infection. Ablating the fetal layer in dirty mice led to a higher pathogen load and was comparable to levels seen in clean mice with a normal T cell compartment. These data indicate early microbial exposure alters susceptibility to disease in adulthood by changing the developmental layering and programming of the CD8+ T cell compartment.

Presenter: Takehara, Kennidy

CD8 T cell residency programs in prostate infection and tumorigenesis

Kennidy Takehara, Miguel Reina Campos, Ananda Goldrath

University of California San Diego

In response to pathogens, CD8+ T cells differentiate and expand into a pool of heterogenous effector cells necessary for pathogen clearance. Thereafter, a select portion of this effector pool is retained as memory T cells that can often persist for the lifetime of the animal. Memory T cell subsets can further be divided by their migration patterns and phenotype, with some memory populations continually circulating through the body, while other subsets become resident within tissues. These CD8+ tissue-resident memory cells (TRM) are important as they act as front-line responders to re-infection and tumorigenesis in non-lymphoid tissues where are poised to rapidly respond and induce a highly specific and potent immune recall response. In recent years, these cells have been thoroughly characterized in various tissues, including the lung, gut, salivary gland, and female reproductive tract, yet these cells have yet to be identified or characterized in the prostate. The prostate acts as a barrier surface to the urinary tract microbiome and prevents infection of the male reproductive system; however, the presence and function of prostate TRM and their role in mitigating inflammation and cancer progression has yet to be elucidated. It has been proposed that prostate inflammation is a risk factor for the development of prostate cancer, a process in which TRM may play a pivotal role. We have developed a novel mouse model utilizing TCR transgenic CD8+ T cells recognizing LCMV gp33 presented by MHC class I and acute systemic infection with the lymphocytic choriomeningitis virus (LCMV) to generate bona-fide prostate TRM. This model will help us identify the regulators of prostate TRM and their potential role in anti-cancer immunity. We find CD8+ T cells within the prostate epithelium up to 3 months following LCMV infection, with a surface expression and transcriptional profiles consistent with a resident memory population. We hypothesize that prostate TRM limit tumorigenesis through mechanism of immune-surveillance in healthy tissue, but could also contribute to worsen inflammatory conditions that lead to cellular transformation. We expect that the biological adaptations of CD8 T cells to prostate residency will unveil a set of mechanisms that can be exploited as immunotherapies.

Presenter: Tan, Wei Hong

A novel cell-extrinsic regulation of NK cell that is cGAS/STING-dependent but IFN-independent.

Wei Hong Tan, Arne M. Knudsen, Tayla Olsen, Jonathan Linton, Allie Kehret, Emily Collins, Anthony Rongvaux

Fred Hutchinson Cancer Research Centre

Natural killer (NK) cells are innate lymphocytes that defend against pathogens and cancers. Although poorly understood, NK cell deficiencies are known to occur in chronic inflammatory diseases and infections. In contrast, DNA-sensing cytosolic receptor cyclic GMP-AMP synthase (cGAS) and its downstream effector, stimulator of interferon (IFN) genes (STING), critically regulate innate immune responses by NK cells against inflammatory diseases and infections. We now report a novel mechanism of cell-extrinsic regulation of NK cell proliferation and activation that is cGAS/STING-dependent but IFN-independent. We observed lower NK cell frequency and maturation in peripheral blood and spleens of mice deficient in proapoptotic caspases 3/7/9; we also measured functional deficiencies that are partially dependent on the cGAS/STING pathway, with reduced IFNγ production and reduced killing in response to foreign and infected cells, respectively. Surprisingly, these responses are type I IFN signaling-independent. Moreover, wild-type NK cells became hyporesponsive upon transfer into caspase-deficient mice, suggesting a cell-extrinsic phenotype. RNAseq analysis revealed that NK cells in caspase-deficient mice have higher expression of EGR1 and EGR2, which are key regulators in T-cell exhaustion. Better understanding the mechanisms behind NK cell deficiency and hypofunction will elucidate the regulation of NK cells in chronic inflammation and infections.

Presenter: Tantin, Dean

Transcription coactivator OCA-B/Pou2af1 is necessary and sufficient to promote T cell-intrinsic CD4 memory

Dean Tantin, Wenxiang Sun, Heejoo Kim, Jelena Perovanovic, Andrea Ibarra, J Scott Hale, Matthew A Williams

University of Utah School of Medicine

The establishment of durable immunological memory remains a poorly understood aspect of lymphocyte biology and a limiting factor in vaccine development. Using a T cell-conditional OCA-B (Pou2af1/Bob.1/OBF-1) knockout model and a natural mouse viral pathogen, we show that expression of the transcription cofactor OCA-B within T cells is necessary for proper CD4+ memory T cell formation. We also show that ectopic OCA-B expression is sufficient to drive T cells towards a memory fate while having no effect on primary antiviral effector response. Bulk and single-cell gene expression profiling comparing cells transduced with OCA-B and empty vector at peak primary viral response identifies changes in gene expression consistent with later memory formation. Computationally intersecting differentially expressed genes in bulk RNA-seq with previously identified targets identifies direct targets both increased (Tbx21, Zeb2, Gadd45b, Ikzf2, Socs2) and decreased (Zbtb16, Ccr1) by ectopic OCA-B expression. Single-cell RNA-seq reveals expansion of short-lived effector T cell compartments with increased expression of Gadd45b and Socs2, as well as increased expression of memory-associated genes such as Slamf6, Bcl2, Il7r and Tcf7 in clusters of effector cells with memory potential. We also describe for the first time the generation of an OCA-B-mCherry reporter mouse that efficiently labels B and T lymphocytes and that shows high expression in CD4+ TCM cells. Cumulatively, the results demonstrate that OCA-B expression in T cells is necessary and sufficient to promote CD4 T cell memory in vivo.

Presenter: Tejeda-Garibay, Susana

Intestinal and tracheal microbiomes inhibit in vitro growth of Coccidioides immitis

Susana Tejeda-Garibay, Anh L. Diep, Katrina K. Hoyer

University of California Merced

Valley fever is a disease caused by Coccidioides, a fungal pathogen, and is frequently misdiagnosed as community acquired bacterial pneumonia and treated with several rounds of antibiotics prior to accurate diagnosis. Antibiotic treatment changes the microbiome repertoire and host immunity; the impact of these microenvironmental changes on Coccidioides invasiveness is unknown. A soil microbial antagonist related to Bacillus subtilis inhibits in vitro growth of Coccidioides immitis through a clear zone of inhibition between fungi and bacteria. Whether host microbiota also could inhibit Coccidioides is unknown. To assess whether the host microbiota has inhibition capabilities against Coccidioides, we performed two types of inhibition assays. A 50/50 inhibition assay was performed in which the host microbiota and Coccidioides were placed in direct competition simultaneously on agar plates. A spike in inhibition assay was also performed in which the host microbiota was allowed to reach ~80% confluency, to mimic an established in vivo microbiome, before spiking in Coccidioides. The area of growth was observed and measured at day 4, 7, and 11. Our in vitro data indicate that specific intestinal and tracheal host microbiome species inhibit Coccidioides growth. To assess if partial in vitro depletion of host microbiota allows greater Coccidioides growth, antibiotic disc diffusion assays were performed. Our data suggests that depleting commensal microbiomes, allows a niche for Coccidioides growth. These in vitro findings could have clinical relevance and shape the way physicians assess prescription of antibiotics and coccidioidomycosis diagnosis. Experiments to identify the inhibitory microbiota and proteomic interactions are ongoing.

Presenter: Tharp, Kevin

Mechano-metabolic programming of macrophages suppresses CD8-mediated anti-tumor immune responses.

Kevin Tharp, Maller O.M., Kersten K., Timblin G.A., Krummel M.F., Weaver V.M.

University of California, San Francisco

Tumor progression is enabled by the failure of the immune system to identify and destroy the oncogenic and metastatic cells comprising the tumor. Inapt macrophage phenotypes can limit productive anti-tumor immune responses. Here, we demonstrate that macrophages responding to the stiff fibrotic tumor microenvironment (TME) initiate a collagen synthesis program associated with wound healing. The collateral effect of this biosynthetic programming is an untenable metabolic milieu for productive anti-tumor responses by cytotoxic CD8 T cells. Collagen synthesizing macrophages consume environmental arginine, synthesize proline, and excrete ornithine that retards CD8 anti-tumor responses. Overall, the data presented here indicate that the fibrotic TME associated immune desert or immune excluded phenotypes may not be due to direct physical exclusion of CD8s, but a secondary effect of mechano-metabolic reprogramming of macrophages in the TME.

Presenter: Thulin, Natalie K.

Defining the contribution of IRF5 signaling to TLR7-driven Macrophage Activation Syndrome

Natalie K. Thulin, Susana L. Orozco, Betsy J. Barnes, Jessica A. Hamerman

University Of Washington

Macrophage Activation Syndrome (MAS) is a serious and potentially fatal complication of rheumatic disease or viral infection. MAS is characterized by the development of cytopenias and accumulation of hemophagocytes— activated macrophages that phagocytose red blood cells (RBCs). I will investigate spontaneous MAS-like disease in a mouse model of Systemic Lupus Erythematosus. This model has overexpression of and constitutively active signaling through the endosomal single-stranded RNA sensor, TLR7 (TLR7.1 mice), which drives chronic inflammation and subsequent MAS disease. TLR7.1 MAS is characterized by thrombocytopenia, anemia, and a novel population of hemophagocytes, inflammatory hemophagocytes (iHPCs), that spontaneously differentiate from Ly6Chi monocytes. In this model, anemia development is positively correlated with iHPC phagocytosis of RBCs indicating that iHPC hemophagocytosis may drive disease. In humans, SNPs in the gene encoding the transcription factor interferon regulatory factor 5 (IRF5) are associated with MAS development. Further, IRF5 signaling is critical for the development of inflammatory macrophages in the context of several inflammatory diseases. Previous work in our lab showed that IRF5 ablation in vivo ameliorates iHPC differentiation downstream of acute TLR7 signaling. However, in the context of in vivo TLR7-driven MAS, the role of IRF5 signaling in iHPC production and MAS disease is unclear. I am determining the contribution of signaling through IRF5 to MAS disease development and iHPC differentiation and function in the TLR7.1 MAS model.

Presenter: Timblin, Greg A.

Mitochondrial CoA redox state governs macrophage inflammatory metabolic flux

Greg A. Timblin, Kevin M. Tharp, Johanna ten Hoeve-Scott, Valerie M. Weaver

UCSF

Host-targeted therapies aimed at reinvigorating anti-tumor and anti-pathogen immunity will only work if immune cells are able to engage the metabolic programs and nutrient fluxes that support anti-tumor/pathogen responses. We demonstrated that the transition of macrophages from a proinflammatory/M1 state to a tolerized/anti-inflammatory/M2 state, which occurs during tumor progression and infection, is a product of mitochondrial oxidative stress resulting from Toll-like receptor (TLR)-driven mitochondrial reactive oxygen and electrophilic species (mtROS/mtRES) production (Timblin Nature Metabolism 2021). However, the mechanism by which this stress alters nutrient flux to limit proinflammatory programming is unclear. We have discovered that mitochondrial oxidative stress disrupts Coenzyme A (CoA) homeostasis by promoting CoAlation, whereby CoA uses its reducing power to act as a mitochondrial antioxidant, protecting mitochondrial proteins from overoxidation by forming mixed disulphides. This creates a "CoA sink" that reduces free CoA available to support glucose entry into the TCA cycle, limiting acetyl-CoA production required for histone acetylation and proinflammatory gene expression. Supporting this model, metabolic tracing experiments demonstrate CoA supplementation restores TLR-driven flux of C13-glucose in the TCA cycle of tolerized macrophages, restoring acetyl-CoA production and TLR-dependent proinflammatory responses. Moreover, in naïve macrophages, CoA supplementation has a strong "priming" effect, boosting the magnitude of TLR-dependent inflammatory responses in vitro and in vivo. Finally, CoA acts as a "metabolic adjuvant", boosting anti-tumor efficacy of a TLR ligand in a murine breast cancer model. This work highlights the importance of considering metabolism and nutrient fluxes in the design of host-targeted therapies aimed at reprogramming immune cell phenotypes.

Presenter: Tomala, Jakub

CYTOKINE-ANTIBODY SINGLE-CHAIN FUSIONS FOR CANCER IMMUNOTHERAPY

Jakub Tomala, Elissa Leonard, Michael Leff, Jamie Spangler

Institute of Biotechnology, Academy of Sciences of the Czech Republic, Prague, Czech Republic
Johns Hopkins University, Baltimore, MD, USA

Interleukin-2 (IL-2) is a multifunctional cytokine that is able to potently stimulate immune effector cells (e.g., CD8+ T and NK cells). Unfortunately, its concurrent promotion of regulatory T cells (Treg) and harmful off-target effects have limited its clinical efficacy. Boyman (Boyman et al., Science, 2006) reveal methods with which to mitigate these issues by complexing mouse IL-2 to anti-IL-2 mAb S4B6. These IL-2 complexes are superior to free IL-2, they manifest selective stimulatory activity for memory CD8+ T and NK cells and possess significant antitumor activity. However, the potential clinical use of these complexes is limited due to the mouse origin of IL-2 and the dissociation of the complexes at low concentrations. Based on our previous studies, we designed, engineered and produced translationally relevant protein chimera (immunocytokine, IC) consisting of hIL-2 linked through a flexible oligopeptide spacer to light chain of anti-hIL-2 mAb MAB602, either in unmodified or mutated version, functionally similar to scIL-2/S4B6 immunocytokine (Tomala et al., ACS Chem Bio, 2013). This approach circumvents disadvantages of IL-2/S4B6 mAb complexes and exerts sufficient biological activity. We demonstrate that this IC we produced contained both IL-2 and mAb in a single molecule and IL-2 interacted with binding site of mAb. We also demonstrate its biophysical characteristics related to IL-2 receptor, its biological activity in vitro and in vivo and also therapeutic potential to eradicate experimental tumors.

Presenter: Tran, Mai

Tracing differences in susceptibility to a naturally occurring enterovirus infection

Mai Tran, James Gagnon, Nels Elde

University of Utah

Viral infection of the same virus strain can result in dramatically different clinical severity due to the ability of some viruses to target more than one host tissue in some but not all patients. The zebrafish, with unrivaled optical accessibility and a suite of well-developed genetic and molecular tools, is a powerful but currently underappreciated model system to study host-virus interactions. Our recent discovery of a naturally occurring enterovirus - Zebrafish picornavirus (ZfPV) - that infects zebrafish provides a new opportunity to study enterovirus-host interactions of an endemic virus. ZfPV infections of the Tübingen strain appear asymptomatic but elicit strong host immune responses in the intestine. In contrast, infection in the CG2 strain results in significantly higher viral load and infection of the CNS. Using single-cell RNA sequencing, we identify specific cell populations infected by ZfPV in the intestines of TU and CG2. Preliminary results suggest that while infection did not lead to increased cell death, genes involved in mitophagy and autophagy activities are upregulated. Additionally, differences in basal interferon activity may underlie differences in susceptibility to infection. Using comparative transcriptomic to investigate host-enterovirus interactions locally and systemically, we will gain a better understanding of cellular mechanisms underlying the observed strain-specific differences in host susceptibility to enterovirus infection.

Presenter: Van Gelder, Rachel

IFN-γ expression is controlled by intron detention

Rachel Van Gelder, Ram Savan
University of Washington

Interferon-gamma (IFNγ) is a cytokine central to immunity against microbial pathogens. Humans and mice lacking IFN-γ are highly susceptible to lethal infection. IFNG mRNA is post-transcriptionally regulated by multiple mechanisms. AU-rich elements in the 3' untranslated region induce transcript degradation to prevent chronic inflammation. Further, several microRNAs and long noncoding RNAs modulate IFN-γ expression. However, known mechanisms of post transcriptional regulation fail to explain an observed phenomenon, in which natural killer (NK) cells harbor formed IFNG mRNA at homeostasis that is exported and translated very rapidly upon additional cytokine stimulus. Intron detention (ID) is a post-transcriptional regulatory event that may lead to retention of unspliced mRNA in the nucleus, delayed mRNA translation or transcriptional decay. We observe that while NK cells treated with IL-12 alone transcribe IFNG, the transcript is relegated in the nucleus with its introns intact. When NK cells are treated with the cytokine IL-2 in addition to IL-12, the IFNG gene is further spliced. IL-2-dependent intron splicing appears to occur in a transcriptionally independent manner. Further, we have preliminary evidence that blocking ERK1/2 signaling downstream of the IL-2 receptor inhibits splicing IL-2 mediated splicing of IFNG mRNA. We therefore propose that ID results in nuclear retention of IFNG mRNA, ensuring that NK and other IFNγ producing cells are poised to robustly and rapidly produce protein after microbial sensing.

Presenter: Viramontes, Karla M.

PD-1 immune checkpoint blockade can synergize with PSGL-1 inhibition to reinvigorate exhausted T cells

Karla M. Viramontes, Emily N. Neubert, Julia M. DeRogatis, and Roberto Tinoco

Department of Molecular Biology and Biochemistry, School of Biological Sciences, University of California Irvine, Irvine

P-selectin glycoprotein ligand-1 (PSGL-1) is an immune checkpoint regulator that is highly expressed on T cells. PSGL-1- deficient mice infected with chronic lymphocytic choriomeningitis virus (LCMV) were shown to control chronic viral infection due to increased effector function by anti-viral T cells. However, the cell-intrinsic role of PSGL-1 expression on CD4+ and CD8+ T cell exhaustion is not fully known. Using adoptive transfers of WT or PSGL-1-deficient TCR transgenic CD4+ and CD8+ T cells in mice infected with chronic LCMV, we characterized the differentiation and effector response. We found increased expansion and effector function by PSGL-1-deficient T cells early during infection, however, at later stages of infection, PSGL-1-deficient T cells were functionally exhausted. We found that exhausted CD4+ and CD8+ PSGL-1-deficient T cells were reinvigorated more effectively than WT cells after PD-L1 blockade due to their increased proliferation, cytokine production, and accumulation. We also observed increased responses of CD8+ PSGL-1-deficient T cells in melanoma tumors. Our findings showed that PSGL-1 expression was required for the maintenance of both CD8+ and CD4+ exhausted T cells and that PSGL-1 deletion synergized with PD-1 blockade to reinvigorate these cells. These findings highlight an important cell-intrinsic role for PSGL-1 expression in the maintenance and reinvigoration of exhausted T cells.

Presenter: Wade-Vallance, Adam K,

B cell receptor stimulation induces IgE plasma cell apoptosis

Wade-Vallance Adam K, Yang Zhiyong, Libang Jeremy, Robinson Marcus J, Tarlinton David M, Allen Christopher DC

University of California, San Francisco

The successful regulation of immunoglobulin E (IgE) production safeguards health from allergic disease, but the mechanisms that restrain the longevity of IgE-secreting plasma cells (PCs) are unknown. Here, we find that IgE PCs have uniquely elevated expression of the B cell receptor (BCR) and are highly sensitive to cognate antigen. After BCR stimulation, IgE PCs phosphorylate intracellular signaling proteins and undergo apoptosis. The extent of IgE PC apoptosis correlates with the strength and duration of BCR stimulation and requires Syk, BLNK, and PLCγ2. In vivo, PC-specific impairment of BCR signaling selectively increases IgE PCs. Conversely, targeting the IgE BCR for ligation using αIgE depletes IgE PCs in an Fc-receptor-independent manner. These findings reveal a putative mechanism of allergic tolerance that may also contribute to the success of allergy treatments that could ligate IgE PC BCRs, such as allergen immunotherapy and αIgE therapeutics (e.g. Omalizumab).

Presenter: Waldman, Monique

Ena/VASP proteins contribute to naïve CD8+ T cell activation and expansion by promoting T-APC interactions in vivo

Monique Waldman, Jeremy T. Rahkola, Benjamin A.S. Willett, Jeffrey W. Chung, Ashton L. Sigler, Rachel S. Friedman, Ross M. Kedl, Jordan Jacobelli

University of Colorado Anschutz

Naïve T cell activation in secondary lymphoid organs such as lymph nodes (LNs) occurs upon recognition of cognate antigens presented by antigen presenting cells (APCs). T cell activation requires cytoskeleton rearrangement and sustained interactions with APCs. Ena/VASP proteins are a family of cytoskeletal effector proteins responsible for actin polymerization and are frequently found at the leading edge of motile cells. Ena/VASP proteins have been implicated in motility and adhesion in various cell types, but their role in primary T cell activation has not been explored. Our goal was to determine the contribution of Ena/VASP proteins to T cell activation and expansion in vivo. Our results showed that naïve T cells from Ena/VASP-deficient mice have a significant reduction in antigen-specific T cell accumulation following Listeria monocytogenes infection. The kinetics of T cell impairment were further confirmed in Ena/VASP-deficient T cells stimulated via dendritic cell immunization. To investigate the cause of this T cell expansion defect, we analyzed T cell-APC interactions in vivo by 2-photon microscopy and observed fewer Ena/VASP-deficient naïve T cells interacting with APCs in LNs during priming. We also found that Ena/VASP-deficient T cells formed conjugates with significantly less actin polymerization at the T cell-APC synapse, and that these conjugates were less stable than their WT counterparts. Thus, we conclude that Ena/VASP proteins contribute to T cell actin remodeling downstream of T-APC interactions required for the initiation of stable T cell conjugates during APC scanning and for efficient activation and expansion of T cells in vivo.

Presenter: Walker, Matthew

Identification and characterization of B cell dependent regulatory T cells

Matthew Walker, Jaime Chao and Peter Savage

University of Chicago

Recognition of self-peptide/MHC-II ligands in the periphery drives clonal regionalization and establishment of unique phenotypes of Foxp3+ T-regulatory (Treg) cells. Here, we tested the hypothesis that a fraction of peripheral Foxp3+ regulatory T cells require intimate interactions with B cells for their thymic development and peripheral homeostasis, and are specialized to regulate self-specific B cells in the periphery. Using deep T cell receptor profiling of Treg cells, we found that mice with restricted B cell diversity exhibited altered Treg cell phenotypes and loss or enrichment of distinct Treg cell clones, suggesting that the diversity of the self-reactive B cell compartment impacts Treg cell repertoire diversity. In addition, we observed that the inducible depletion of B cells led to a significant loss of polyclonal Treg cells. Ongoing work is focused on elucidating the biology of putative B cell dependent Treg cells at the clonal level, at both steady state and following immunization with foreign antigen. Together, our observations suggest the existence of a specialized B cell – Treg cell axis that may play unique roles in immune regulation.

Presenter: Wang, Jessica (Jiaxi)

Single-cell multiomics defines tolerogenic extrathymic Aire-expressing cells with unique homology to thymic epithelium

Jessica (Jiaxi) Wang, Caleb A. Lareau, Jhoanne L. Bautista, Hong Sun, Alexander R. Gupta, James M. Gardner

UCSF

The autoimmune regulator (Aire) protein, a key transcriptional regulator expressed in medullary thymic epithelial cells (mTECs), is crucial for central tolerance by inducing tissue specific antigen (TSA) expression in mTECs. Interestingly, Aire is also found in extrathymic Aire-expressing cells (eTACs) in secondary lymphoid organs. We found that eTACs are hematopoietic antigen-presenting cells that are capable of inducing immune tolerance, but the precise identity of eTACs has remained unclear. To define eTACs at the transcriptional, genomic, and proteomic level, we utilized a combination of single-cell multiomics, transgenic murine models, and functional approaches. We found that eTACs consist of two similar cell types: CCR7+ Aire-expressing migratory dendritic cells (AmDCs) and an Aire-high population co-expressing Aire and retinoic acid receptor–related orphan receptor γt (RORγt) that we termed Janus cells (JCs). Both JCs and AmDCs are transcriptionally and genomically most similar to migratory dendritic cells. eTACs, particularly JCs, have highly accessible chromatin and share remarkable homology with mTECs, including their RANK-dependent Aire expression. Additionally, transgenic self-antigen expression by eTACs is sufficient to induce negative selection of cognate autoreactive T cells and prevent autoimmune diabetes. The transcriptional, genomic, and functional symmetry between eTACs and mTECs potentially identifies a core program driven by Aire that may influence self-representation and tolerance across the spectrum of immune development. To better decipher the function of extrathymic Aire in eTACs and its contribution to peripheral immune tolerance, we have generated a Peripheral Aire KnockOut (PAKO) mouse by crossing Vav1-Cre with Aire-fl/fl mice. Using intracellular Aire staining by flow cytometry, we validated that PAKO mice lack all eTACs in secondary lymphoid organs while maintaining normal thymic Aire expression in mTECs. Studying the biology of eTACs and peripheral Aire will help further elucidate the function of Aire and define basic peripheral tolerance mechanisms, which may have significance for a range of clinical applications from autoimmunity to tumor immunity to maternal-fetal tolerance.

Presenter: Watson, Robert O.

Mitochondrial dysfunction promotes alternative gasdermin D-mediated inflammatory cell death and susceptibility to infection

Robert O. Watson(1), Chi G. Weindel(1), Xiao Zhao(2), Eduardo Martinez(1), Samantha L. Bell(1,3), Krystal J. Vail(4,5), Aja K. Coleman(1), Jordyn J. VanPortfliet(1), Baoyu Zhao(6), Cory J. Mabry(1), Pingwei Li(6), A. Phillip West(1), Jason Karpac(2), Kristin L. Patrick(1), Robert O. Watson(1)**

(1)Department of Microbial Pathogenesis and Immunology, Texas A&M University, College of Medicine, TX, 77807, USA (2)Department of Molecular and Cellular Medicine, Texas A&M University, College of Medicine, TX, 77807, USA (3)Department of Microbiology, Biochemistry & Molecular Genetics, Rutgers New Jersey Medical School, NJ, 07103, USA (4)Department of Veterinary Pathobiology, Texas A&M University College of Veterinary Medicine and Biomedical Sciences, TX, 77843, USA (5)Division of Comparative Pathology, Tulane National Primate Research Center, Covington, LA 70433, USA (6)Department of Biochemistry and Biophysics, Texas A&M University, 77843, USA; *co-corresponding

Human mutations in mitochondrial-associated genes are linked to inflammation and susceptibility to infection. However, their mechanistic contributions to immune outcomes remain ill-defined. We discovered the disease-associated gain-of-function allele Lrrk2G2019S (leucine-rich repeat kinase 2) perturbs mitochondrial homeostasis and reprograms cell death pathways in macrophages. In the presence of Lrrk2G2019S-dependent mitochondrial stress, AIM2 inflammasome activation promotes more cell death but not more pyroptotic IL-1β release. Instead, inflammasome activation triggers gasdermin D-mediated mitochondrial pores, driving ROS-mediated RIPK1/RIPK3/MLKL-dependent necroptosis. Consistent with this, infection of Lrrk2G2019S mice with Mycobacterium tuberculosis elicits hyperinflammation and immunopathology via enhanced neutrophil infiltration. Our findings suggest a pivotal role for gasdermin D as an executer of multiple cell death pathways and demonstrate that mitochondrial dysfunction can direct immune outcomes via cell death modality switching. This work provides insights into how LRRK2 mutations manifest or exacerbate human diseases and identifies GSDMD-dependent necroptosis as a potential therapeutic target to limit Lrrk2G2019S-mediated immunopathology.

Presenter: Weckel, Antonin

Early life interactions between commensal bacteria and dermal CD301b+ cDC2s facilitate long-term immune tolerance to the skin microbiome

Antonin Weckel, Julianne Riggs, Geil Merana, Jeanmarie Gonzalez, Joy Okoro, Miqdad Dariwhala, Yosuke Kumamoto , Tiffany Scharschmidt

University of California San Francisco

Tolerance to commensal bacteria is critical for skin immune homeostasis. We have previously shown that tolerance to skin commensals is preferentially established in neonatal life and supported by generation of commensal-specific regulatory T cells (Tregs). Here, we set out to identify how dendritic cell (DC) interactions with bacteria in neonatal skin facilitate commensal-specific Treg formation. Colonization of neonatal mice with fluorescent Staphylococcus epidermidis (SE) demonstrated that type 2 conventional DCs (cDC2s), particularly those expressing the C-type lectin CD301b, are the primary DC subset to phagocytose and traffic SE to the skin-draining lymph node. CITE-seq revealed that CD301b marks a subset of dermal cDC2s enriched for phagocytic and antigen presentation pathways. Notably, SE phagocytosis prompts neonatal CD301b+ cDC2s to increase expression of both maturation and regulatory molecules indicative of a mature regulatory DC (mregDC) program, which has been linked to Treg generation in other contexts. Using in vitro SE-DC-T cell co-cultures, we find that CD301b+ cDC2s preferentially drive commensal-specific CD4+ proliferation and Treg generation. Depleting this subset in neonatal Mgl2DTR mice substantially prevented accumulation of SE-specific Tregs in vivo. When CD301b+ DCs are transiently depleted during the neonatal period, reduction of the SE-specific Treg compartment persists into adulthood, and adult re-exposure to SE in the context of skin barrier disruption causes heightened Th17-polarized skin inflammation. Lastly, we utilize a novel human skin explant system that enables bacterial colonization of human foreskin to demonstrate that SE colonization also causes human skin cDC2s to adopt an mregDC phenotype. Taken together, our results identify CD301b+ cDC2s and their polarization into mreg DCs as critical for the neonatal establishment of long-term tolerance to skin commensals.

Presenter: Wheeler, Benjamin

Malat1 and miR-15/16 constitute a non-coding RNA circuit that regulates cytotoxic T cell biology

Benjamin Wheeler, John Gagnon, Darryl Mar, Didi Zhu, Priscila Muñoz-Sandoval, Alex Marson, Mark Ansel

UCSF

Precision control of cytotoxic T cell activation, function, and differentiation is essential for adaptive immune responses to viruses and cancer. Here we demonstrate a novel interaction between the lncRNA Malat1 and the miR-15/16 microRNA family. Through miR-15/16 Malat1 controls down stream miR-15/16 targets. In particular, CD28's expression and function in co-stimulating CD8 t cells is regulated by this circuit. miR-15/16 is known to control CD8 T cell responses to LCMV infection. Here we further show Malat1 also controls these responses in the acute and memory phase of infection in a reciprocal manner to miR-15/16. Our on going and future works seeks to extend these results to anti-cancer immunity. In that context this circuit or its direct protein-coding targets may serve as novel ways in which to tune T cells responses particularly in CAR-T cells and other adoptive transfer therapies.

Presenter: Wu, Ting-Ting

Virus-like Vesicles as a Vaccine Strategy for Kaposi-sarcoma Associated Herpesvirus

Ting-Ting Wu, Alex K. Lam, Claire Harelson, Danyang Gong, Xi Ma, Khalid Rashid, Gurpreet Brar, Ren Sun, Caius Radu

UCLA

The Kaposi Sarcoma-associated Herpesvirus (KSHV) is a tumor associated virus that is linked to various malignancies. A prophylactic vaccine against KSHV would prevent or reduce the occurrence of diseases such as Kaposi Sarcoma and Primary Effusion Lymphoma. However, a commercial vaccine is still not available. Our laboratory has developed a method to generate virus-like vesicles (VLVs) of KSHV. VLVs present the repertoire of viral glycoproteins but are noninfectious due to a deficiency in capsid formation. VLVs are able to stimulate the innate immune system, and we have demonstrated that immunization of VLVs with DNA- or lipid nanoparticle-based adjuvants results in the generation of virus specific T cells and antibodies. Importantly, these antibodies have neutralizing ability, which is enhanced in the presence of complement. Overall, VLVs provide a platform for the development of anti-viral immunity for a KSHV vaccine

Presenter: Wu, Yixuan

Distal cutaneous immunization triggers a heightened alert state in the intestinal immune system.

Yixuan Wu, Meera K Shenoy, Jeanette Schwensen, Michael T Conlon, Meghan A Koch, Michael Gerner

University of Washington

Conventionally, immune responses are studied in the context of local inflamed tissues or the corresponding draining lymph nodes (LNs). However, little is known about whether inflammatory signals can act on distal tissues and non-draining LNs. Using a mouse model of cutaneous immunization with various Toll-like receptor (TLR) agonists, we found that in addition to local skin-draining LNs, there was a marked distal immunological response in the small intestine and the mesenteric LN (mLN) that was absent in other barrier tissues. Specifically, we observed rapid vascular leakage and delivery of systemic antibodies into both the lamina propria of the small intestine and the draining mLN, as well as activation and migration of intestinal dendritic cells to the mLN. These inflammatory processes were in part dependent on TNFa signaling and returned to homeostasis within 48 hours, suggesting a transient intestine-specific alert state caused by systemic inflammation. Furthermore, in a mouse model of enteropathogenic and enterohemorrhagic E. coli (EPEC, EHEC) infection with the oral pathogen Citrobacter rodentium, cutaneous adjuvant administration enhanced the protective effect of passive immunization while also increasing IgG levels in the colonic epithelium. Together, these findings reveal that the intestinal immune system is rapidly and transiently activated in response to systemic inflammation, suggesting the existence of a defense mechanism to enhance rapid surveillance of the intestinal barrier, which is at constant risk of pathogen encounter.

Presenter: Xie, Markus

A novel agonistic anti-SIRP[a] antibody ameliorates mouse arthritis through selective depletion of tissue inflammatory macrophages

Markus Xie, Tianhe Sun, Juan Zhang, Wyne Lee, Rajita Pappu, Tangsheng Yi

Genentech Inc.

Sirpα is an inhibitory immune receptor highly expressed on myeloid immune cells, and interaction of Sirpα with its ligand CD47 mediates a “don’t eat me” signal to inhibit myeloid cell phagocytosis and aberrate activation. Here we report the discovery of a novel Sirpα agonist antibody. The crosslinking of this agonist antibody potently inhibits antibody dependent macrophage activation through repressing TNF and IL-1 production. Sirpα agonist antibody reduces Zymosan- or LPS- induced inflammatory macrophage accumulation. It selectively depletes monocyte derived macrophage through promoting apoptosis without affecting tissue resident macrophage or neutrophils. Sirpα agonist antibody significantly ameliorates KBxN serum induced arthritis and collagen induced arthritis. Amelioration of arthritis by Sirpα agonist antibody is associated with reduced monocyte derived macrophage in the joint and repressed tissue inflammatory cytokines. Collectively, our results provide a therapeutic rationale for using an agonistic anti-Sirpα antibody for autoimmune joint inflammation through the elimination of tissue inflammatory macrophages.

Presenter: Xu, Ziyan

Metabolic and functional reprogramming of tumor-associated macrophages via lipid receptor CD36

Ziyan Xu, Shihao Xu, Susan Kaech

The Salk Institute for Biological Studies, University of California San Diego

Macrophages are highly plastic cells of innate immune system with diverse functions in host pathogen defense and regulation of tissue homeostasis. Despite their relative abundance in the tumor microenvironment (TME), the exact function of tumor-associated macrophages (TAMs) and how they are regulated in TME remain largely unknown. Macrophages are also key players in various metabolic processes including lipid metabolism and its dysregulation can result in perturbed macrophage functions and pathologies such as atherosclerosis. Since TME has been characterized as a “lipid-rich” environment by various recent studies, we are interested in understanding how lipids in TME regulate TAMs' metabolism and their pro- or anti-tumor functions. In this research, we firstly characterized the lipid metabolic phenotype of TAMs in several mouse tumor models. We found that tolerogenic subset of TAM (marked by high expression of F4/80 and PD-L1) is highly lipid-laden and has greater ability to import lipids comparing to other subsets. We also found F4/80hi TAMs highly express scavenger receptor CD36 which mediates uptake of oxidized low density lipoprotein (oxLDL) from TME. Using germ line knockout (Cd36-/-) and myeloid-specific knockout (Cd36flox/flox x Csf1r-Cre) mouse models, we observed that binding and uptake of oxLDL by TAMs was significantly blocked, along with decreased expression of immunosuppressive molecules PD-L1 and CD206, and elevated secretion of TNF and type I interferons by CD36-KO TAMs. Altogether, this reprogrammed TAM functional state mediated by CD36 knockout leads to slower tumor growth in a partial T-cell dependent manner. We further conducted single-cell RNA sequencing on CD36-KO and wild-type TAMs and found several pathways including type-I interferon response were up-regulated in CD36-KO TAMs, indicating an anti-tumor functional state. In summary, our data suggests that lipid receptor CD36 plays crucial roles in functional reprogramming of tumor-infiltrating macrophages and inhibition of CD36 may have beneficial effects for cancer immunotherapy.

Presenter: Yang, Chao

Costimulation of TLR8 responses by CXCL4 in Human Monocytes Mediated by TBK1-IRF5 Signaling and Epigenomic Remodeling

Chao Yang, Mahesh Bachu, Caroline Brauner, Ruoxi Yuan, Yong Du, Marie Dominique Ah Kioon, Giancarlo Chesi, Franck J. Barrat, Lionel B. Ivashkiv

Hospital for Special Surgery

CXCL4 regulates responses of immune cells to endosomal TLRs and has been implicated in the pathogenesis of inflammatory and fibrotic diseases. However, mechanisms by which CXCL4 modulates TLR responses, and its functions in monocytes/macrophages, are still unclear. Here we report that CXCL4 changes the profile of the TLR8 response in human monocytes by selectively and dramatically amplifying inflammatory gene transcription and IL-1 production while partially attenuating the IFN response. Mechanistically, costimulation by CXCL4 and TLR8 synergistically activated TBK1/IKK and repurposed these kinases towards an inflammatory response via coupling with IRF5, and by activating the NLRP3 inflammasome without the need for a second signal. CXCL4 strongly induced chromatin remodeling in a cooperative and synergistic manner with TLR8 signaling, inducing de novo enhancers associated with inflammatory genes. These findings identify signaling and epigenomic mechanisms that underly synergistic activation of inflammatory genes by CXCL4 and TLR8, provide a new paradigm for modulation of TLR responses that is relevant for cytokine storm, and suggest targeting the TBK1/IKK-IRF5 axis may be beneficial in inflammatory diseases.

Presenter: Yu, Bingfei

ENTER-seq: single-cell mapping of antigen specificity, immune repertoire and cell fate

Bingfei Yu, Quanming Shi, Julia A. Belk, Kathryn E. Yost, Kevin R. Parker, Huang Huang, Daniel Lingwood, Mark M. Davis, Ansuman T. Satpathy, Howard Y. Chang

Stanford University

Resolving immune receptor-antigen interactions, especially linking antigen specificity to immune repertoires and cell states are essential to understand how antigen recognition drives immune cell fate decisions. Here we describe a technology for lentiviral-mediated cell entry by engineered receptor-ligand interaction (ENTER) to decode receptor-ligand interactions. Engineered lentiviral particles display user-defined ligands on viral surface and deliver fluorescent proteins into target cells upon cognate receptor-ligand interaction. We optimize ENTER to decode interactions between TCR-MHC peptide, BCR-antigen, and other receptor-ligand pairs. Single-cell readout of ENTER by RNA sequencing (ENTER-seq) enables multiplexed enumeration of antigen specificity, TCR clonality, and cell state of individual T cells. ENTER-seq of patient blood samples after CMV infection reveals the viral epitopes that drive human effector memory T cell differentiation and inter-clonal phenotypic diversity of T cells that target the same epitope. ENTER-seq enables systematic discovery of receptor specificity and linkage to cell fates.

Presenter: Zhen, Anjie

Induction of Autophagy reduces IFN-I mediated Inflammation and restores anti-HIV-1 T Cell response in vivo

Wenli Mu[a,b], Valerie Rezek[a,b], Heather Martin[a,b], Mayra A. Carrillo[a,b], Shallu Tomer[a,b], Philip Hamid[a,b], Miguel Lizarraga[a,b],
Otto O. Yang[b,c,d], Beth Jamieson[a], Scott G. Kitchen[a,b,†] and Anjie Zhen [a,b,†]
[a]Division of Hematology/Oncology, Department of Medicine, David Geffen School of Medicine at UCLA,
[b]UCLA AIDS Institute and
the Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, David Geffen School of Medicine at UCLA,
[c]Division of Infectious Disease, David Geffen School of Medicine at UCLA

A hallmark of HIV-1 infection is chronic inflammation. Chronic immune activation drives the pathogenesis of HIV-1 infection, leading to loss of CD4+ T cells and exhaustion of antiviral cellular immunity. Previously, we demonstrated that persistent inflammation mediated by type I interferon (IFN-I) signaling drives T cell exhaustion. Combination of anti-retroviral therapy (ART) and IFN-I receptor blockade therapy led to accelerated viral suppression and reduced latent HIV-1 reservoirs, suggesting that targeting type I IFN signaling can be used as a therapeutic strategy to alleviate T cell exhaustion. However, as IFN-Is are key regulators for antiviral immunity, more specific interventions to fine-tune IFN-I signaling and chronic inflammation are needed. Autophagy is a homeostatic mechanism for disposal of damaged cellular organelles and elimination of intracellular pathogens, which is pivotal for cellular homeostasis, T cell development and function. Autophagy is also impaired during HIV-1 infection. Here we demonstrate that autophagy is directly linked to IFN-I signaling. Impairment of autophagy leads to accumulation of damaged mitochondria and spontaneous IFN-I signaling. Autophagy inducers reduce IFN-I signaling in activated macrophages and restore functions of exhausted anti-HIV-1 T cells in vitro. Importantly, autophagy inducer treatment in HIV-1 infected humanized bone marrow/liver/thymus (BLT) mice significantly reduced persistent IFN-I signaling and immune activation, restored exhausted antiviral T cell responses, and accelerated viral suppression by ART. Autophagy inducer treatment also led to reduced viral rebound after ART withdrawal. Taken together, our data suggest that therapeutically targeting autophagy is a promising approach for treating persistent immune activation and improve immune control of HIV replication.

Presenter: Zheng, Ye

Glucocorticoid signaling and regulatory T cell collaborate to maintain the hair follicle stem cell niche

Ye Zheng, Zhi Liu, Xianting Hu, Yuqiong Liang, Jingting Yu, Huabin Li, Maxim N. Shokhirev

Salk Institute

The maintenance of tissue homeostasis in steady state or under stress is dependent on the proper communication between the stem cells and the supporting cells in their microenvironment or “niche”. In addition to promoting immune tolerance, regulatory T cells (Tregs) have recently emerged as a critical component of the stem cell niche in the hair follicle (HF), injured muscle, bone marrow, and small intestine to support stem cell differentiation or maintain their quiescence. How Treg cells sense the dynamic signals in the niche environment and communicate with stem cells during tissue regeneration is largely unknown. Here, by using HF as a model, we uncover a hitherto unrecognized function of steroid hormone glucocorticoid that instructs skin-resident Treg cells through glucocorticoid receptor (GR) to facilitate hair follicle stem cell (HFSC) activation and HF regeneration. Ablation of GR signaling in Tregs blocked depilation-induced hair regeneration and natural hair growth without affecting Treg’s immune suppressive function. Mechanistic study revealed that GR signaling induces skin-resident Tregs to produce TGF-b3, which directly activates Smad2/3 in HFSCs and facilitates HFSC activation and proliferation. Our study identifies a novel crosstalk between skin-resident Tregs and HFSCs mediated by the GR/TGF-b3 axis, highlighting a new avenue to manipulate Tregs to support tissue regeneration.