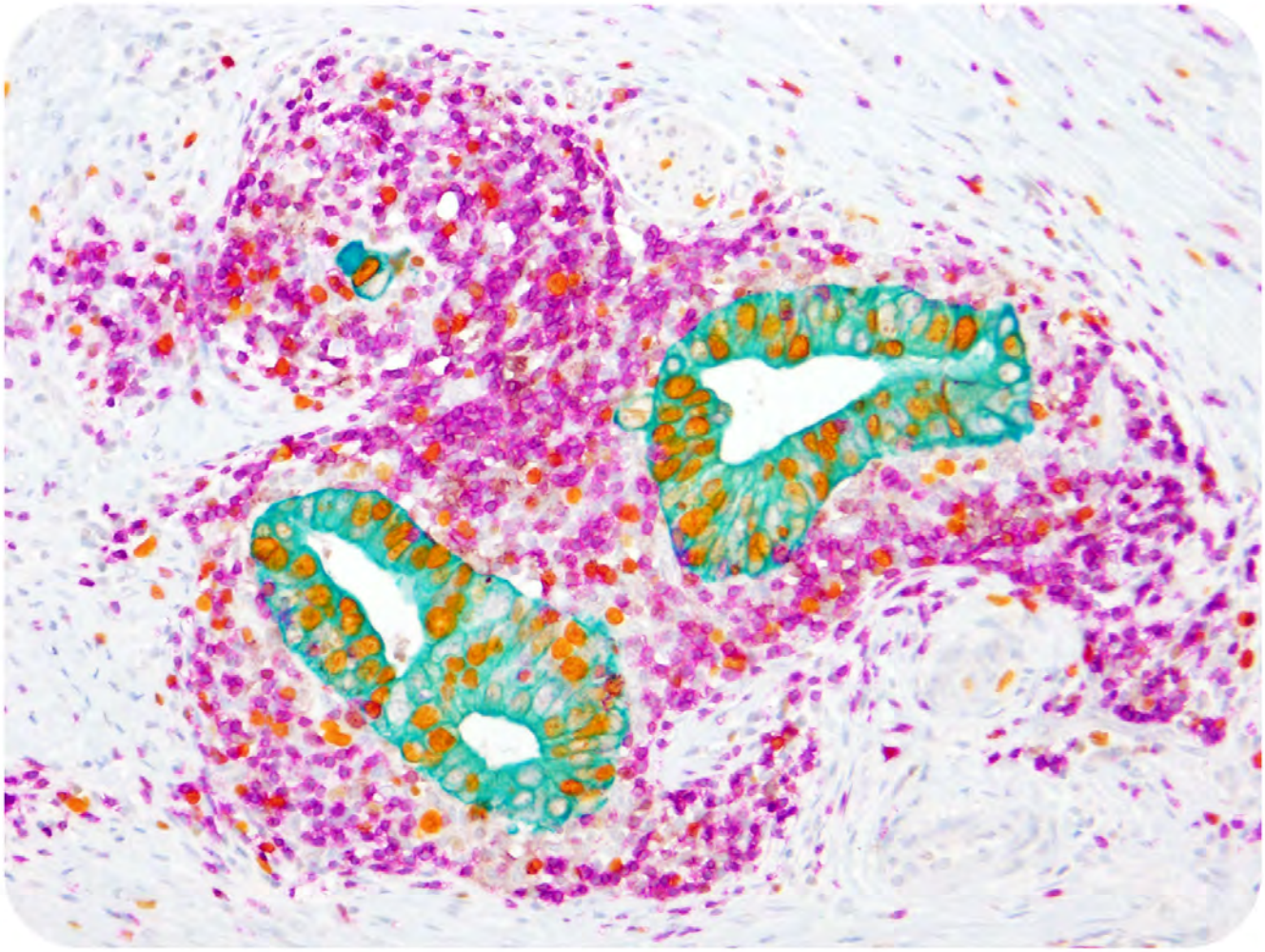
UNIVERSITY *of* PENNSYLVANIA

31st Annual Immunology Graduate Group Retreat

November 2 - 4, 2018 | Lancaster, PA



Locations

Lancaster Marriott at Penn Square
25 South Queen Street
Parking: 38 South Duke Street

The Hotel Lancaster (Overflow hotel)
26 East Chestnut Street
Parking: 150 North Duke Street

Barbaret Bistro & Bakery (Friday reception)
26 East King Street

Lancaster Central Market (Saturday lunch)
23 North Market Street

The Hamilton Club (Saturday dinner and reception)
106 East Orange Street

Friday, November 2

11:00 AM – 12:30 PM **Registration and Lunch** *Heritage Ballroom*

12:30 – 12:40 PM **Welcome** *Heritage Salon C*

Bruce Freedman, VMD, PhD
Associate Professor, Department of Pathobiology
Michael May, PhD
Associate Professor, Department of Biomedical Sciences

12:40 – 2:30 PM **Session I** *Heritage Salon C*
Lymphocyte Development & Differentiation

S1A. CHAIR: Rebecca Rosenthal (Mentor: Michael Cancro, PhD)
"T-bet expression marks a tissue restricted memory B cell subset"

S1B. Thomas Burn (Mentor: Edward Behrens, MD)
"Naturally occurring alternative RAG1 isoforms unveil a novel role for RAG1 N-terminal regions in thymocyte development and negative selection"

S1C. Laura Chopp (Mentor: Rémy Bosselut, MD, PhD)
"CD4 T cell specification in the thymus is independent of the transcription factor Thpok"

S1D. Tanner Robertson (Mentor: Janis Burkhardt, PhD)
"ERM family proteins are required to maintain cortical integrity during S1P-dependent T cell egress"

S1E. Michael Werner (Mentor: Gerd Blobel, MD, PhD)
"Dissection of chromatin reader function in transcription using chimeric BET proteins"

2:30 – 2:45 PM **Break** *Heritage Lobby*

2:45 – 3:45 PM **Session II** *Heritage Salon C*
Immune Mechanisms of Disease

S2A. CHAIR: Brenal Singh (Mentor: Taku Kambayashi, MD, PhD)
"Allergic airway inflammation and airway hyperresponsiveness are independently controlled by diacylglycerol kinase"

S2B. Alexis Crockett (Mentor: Jorge Alvarez, PhD)
"The role of tissue barriers in the neuroimmune mechanisms of schizophrenia"

S2C. Kelly Zullo (Mentor: De'Broski Herbert, PhD)
"Lingo3 interacts with TFF2 to control mucosal integrity, Type 1 inflammation, and colitic tissue repair"

3:45 – 4:00 PM **Break** *Heritage Lobby*

Friday, November 2

4:00 – 5:30 PM	**Poster Pitch Blitz**	*Heritage Salon C*
5:30 – 7:00 PM	**Dinner**	*Heritage Salon AB*
7:00 – 8:00 PM	**New Faculty Talks** Mayaan Levy, PhD Assistant Professor, Department of Microbiology "Intestinal Epithelial Cell Functions in Host-Microbiome Interactions" Michela Locci, PhD Assistant Professor, Department of Microbiology "T follicular helper cell differentiation and functional complexity"	*Heritage Salon C*
8:00 – 9:00 PM	**Keynote** **Thomas Gajewski, MD, PhD** AbbVie Foundation Professor of Cancer Immunotherapy The University of Chicago "Tumor and host factors regulating anti-tumor immunity and immunotherapy efficacy"	*Heritage Salon C*
9:00 PM – 12:00 AM	**Reception**	*Barbaret Bistro*

Saturday, November 3

7:30 – 9:00 AM	**Breakfast**	*Heritage Salon AB*
9:00 – 10:25 AM	**Session III** **Host Immunity to Pathogens** S3A. CHAIR: Megha Basavappa (Mentors: Sara Cherry, PhD and Jorge Henao-Mejia, MD, PhD) "Characterizing the role of long noncoding RNAs in innate antiviral immune responses" S3B. Alexandra A. DeLaney (Mentor: Igor Brodsky, PhD) "Caspase-8 promotes c-Rel-dependent inflammatory cytokine expression and resistance against *Toxoplasma gondii*" S3C. Valeria M. Reyes Ruiz (Mentor: Sunny Shin, PhD) "Detection of the bacterial Type III Secretion System and Flagellin by the human NAIP/NLRC4 inflammasome" S3D. Kelly Rome (Mentor: Warren Pear, MD, PhD) "Trib1 controls antiviral immunity by restraining CD4 and CD8 T cell effector responses during chronic infection"	*Heritage Salon C*
10:25 – 10:40 AM	**Break**	*Heritage Lobby*

Saturday, November 3

10:40 AM – 12:00 PM	**Session IV** **Tumor Immunity**	*Heritage Salon C*
	S4A. CHAIR: Jennifer Wu (Mentor: E. John Wherry, PhD) "The cellular identity of exhaustion: lessons from an in vitro model"	
	S4B. Samir Devalaraja (Mentor: Malay Haldar, MD, PhD) "Role of Retinoic Acid on Mononuclear Phagocytes in the Sarcoma Microenvironment"	
	S4C. Lexus Johnson (Mentors: Andy Minn, MD, PhD / Carl June, MD) "Intratumoral Immune Activation Informs Rational CAR T Cell Design"	
12:00 – 2:00 PM	**Lunch and Free Time**	*Central Market*
12:00 – 2:00 PM	**NIH Lunch**	*Chestnut Boardroom*
2:00 – 3:30 PM	**Alumni Career Panel** (trainees only)	*Heritage Salon DE*
	Stanley Adoro, PhD Assistant Professor, Department of Pathology Case Western Reserve University School of Medicine	
	Alan Copenhaver, PhD Scientist I, Immunobiologics Takeda Pharmaceutical	
	Vered Gigi, PhD Vice President of Strategy and Business Development CURE Pharmaceutical	
	Amy Yellen-Shaw, PhD Medical Writer (Freelance)	
3:30 – 4:30 PM	**New Faculty Talks**	*Heritage Salon C*
	Michael Abt, PhD Assistant Professor, Department of Microbiology "Immune-Microbiota Interactions mediate defense against Clostridium difficile"	
	Ivan Maillard, MD, PhD Professor, Department of Medicine "New roles for Notch signaling in immune regulation"	
4:30 – 6:00 PM	**Poster Session / Happy Hour** Even-numbered posters presented 4:30–5:15 pm Odd-numbered posters presented 5:15–6:00 pm	*Heritage Lobby*

Saturday, November 3		
6:00 – 7:00 PM	**Keynote** **Andrea Cerutti, MD** Professor, Department of Medicine Icahn School of Medicine at Mount Sinai, Catalan Institution for Research and Advanced Studies "New insights into mucosal IgD responses"	*Heritage Salon C*
7:30 – 9:00 PM	**Dinner and Awards**	*Hamilton Club*
9:00 PM – 12:00 AM	**Reception**	*Hamilton Club*
Sunday, November 4		
8:00 – 9:30 AM	**Breakfast**	*Heritage Salon DE*
11:00 AM	**Checkout**	

S1A. "T-bet expression marks a tissue restricted memory B cell subset"

Rebecca Rosenthal*, Arpita Myles*, Joel R. Wilmore, Shannon R. Christensen, Norbert Pardi, Scott E. Hensley, Drew Weissman, Michael P. Cancro

*These authors contributed equally to the work presented.

T-bet$^+$ B cells are a memory B cell subset that enlarges with age and is associated with both viral immunity and humoral autoimmunity. Surprisingly, following PR8 influenza infection, antigen-specific T-bet$^+$ B cells are largely restricted to the spleen and are entirely absent from the draining lymph nodes. To address the question of whether T-bet$^+$ B cells are a splenic resident subset, we parabiosed B6 or T-bet reporter mice 40+ days post PR8 infection with naïve B6.SJL congenic partners. We observed that while PR8-HA specific T-bet$^-$ B cells achieve significant mixing in parabiotic pairs, T-bet$^+$ PR8-HA specific B cells remain in the spleen of the infected partner and are not detected in the lungs, lymph nodes and spleen of the naïve conjoined mouse, showing that the PR8-HA+ T-bet$^+$ B cells are tissue resident in the spleen. Using T-bet reporter mice, we further show that while IgD$^-$ B cells (not antigen-specific) expressing low levels of T-bet are found in the blood of reporter mice and are detectable in the blood of the B6.SJL partner following parabiosis; they are excluded from the spleen and lymphatics. While the origin and fate of these blood T-bet$^+$ B cells remains unclear; our parabiosis study shows that they do not home to the spleen or lymphatics, but persist following splenectomy of previously vaccinated mice. Furthermore, we have recently shown that T-bet$^+$ B cells can be found in the lamina propria of reporter mice and these cells may represent a distinct T-bet$^+$ B cell population as they show an isotype profile distinct from that observed in Tbet$^+$ splenic B cells and persist following splenectomy.

S1B. "Naturally occurring alternative RAG1 isoforms unveil a novel role for RAG1 N-terminal regions in thymocyte development and negative selection"

Burn, TN, Arya R, Bassing C, Behrens, EM

The role of RAG1 in lymphocyte development has been extensively studied with respect to genomic rearrangement of lymphocyte receptors. However, more recently, RAG1 has been shown to have functions in addition to VDJ recombination. This idea was spurred from the identification of mutations in Ommen's Syndrome patients that lead to truncated RAG1 products lacking N-terminal, non-core regions of RAG1 that are not necessary for VDJ recombination. While VDJ recombination is sub-optimal in the absence of full-length RAG1, it is not known why Ommen's Syndrome patients exhibit autoimmunity, suggesting a failure in either central or peripheral tolerance. Our identification of a novel nonsense mutation in RAG1 which leads to premature translation termination unveiled the existence of previously undescribed RAG1 isoforms in both mutant and wild-type settings through a unique mechanism of alternative protein translation. Additionally, thymocytes lacking full-length RAG1 exhibit defective negative selection in a super-antigen mediated system. The region of RAG1 that is required for normal negative selection was further refined to an E3-ligase RING domain, as negative selection was also defective in mice with a point mutation that inactivates this E3-ligase activity. Finally, we show that the presence of a

functional E3 ligase domain in RAG1 is necessary for the stability of the important signaling proteins Syk and ZAP70 in developing thymocytes. Because TCR signal strength determines whether a developing T cell will be negatively selected, the levels of these signaling proteins are tightly linked to the outcome of selection, and subsequently the likelihood of escape of auto-reactive T cells.

S1C. "CD4 T cell specification in the thymus is independent of the transcription factor Thpok"

Laura Chopp, Brian Larsen, Avinash Bhandoola, Rémy Bosselut

Helper T lymphocytes (CD4+) develop in the thymus from MHC Class II signaled CD4+CD8+ (double positive, DP) precursor cells. In contrast, CD8+ cytotoxic T cells develop from MHC Class I signaled DP cells. CD4+ T cells are critical to adaptive immunity, yet the molecular mechanisms underpinning their development remain incompletely understood. Commitment to the CD4 lineage requires the transcription factor Thpok, which represses expression of genes specific of the CD8 lineage; however, it is not clear to which extent, if any, Thpok contributes to initiating expression of CD4-lineage genes. To address this question, we sought to identify genes specifically expressed and loci specifically open in MHC II-signaled thymocytes, and determine whether or not they are Thpok-dependent. We utilized RNA-sequencing to define the gene expression program of MHC II-restricted cells at multiple stages of intrathymic development, and ATAC-sequencing to identify regions of open chromatin unique to MHC II- restricted cells. We found that a CD4-lineage "specification" program is initiated in class II signaled cells, and that this program is largely independent of Thpok expression, as most genes upregulated in Thpok-sufficient MHC II-signaled cells are upregulated normally in their Thpok-deficient counterparts. These data support a Thpok-independent specification step of CD4 T cell development. Additionally, these results have defined candidate transcription factors expressed specifically in MHC II-signaled cells, the function of which we are analyzing by gene knock-out and overexpression approaches.

S1D. "ERM family proteins are required to maintain cortical integrity during S1P-dependent T cell egress"

Robertson TF, Burkhardt JK

ERM family proteins link the plasma membrane to the actin cortex in a regulated manner. They influence a wide variety of cellular processes, including migration. Humans with mutations in the ERM protein moesin present with severe combined immunodeficiency characterized by sharp reductions in circulating lymphocytes, despite indications that hematopoietic development is normal. To address the role of ERM proteins in T cells, we generated a novel mouse in which mature T cells lack both ezrin and moesin, the two family members normally expressed in lymphocytes. Similar to human patients, these double knockout (DKO) mice display grossly normal T cell development, but a paucity of T cells in the blood. However, these mice have elevated T cell numbers in the spleen, pointing to a problem in trafficking rather than survival. Competitive *in vivo* migration experiments revealed that ERM-deficient T cells possess a cell-intrinsic defect in egress from secondary lymphoid organs. We optimized a procedure for studying the critical egress signal sphingosine-1-phosphate (S1P) *in vitro* and found that DKO T cells have selective transmigration defect towards S1P, despite normal S1PR1 expression, endocytosis, and S1P-triggered actin polymerization. Live video microscopy shows that WT T cells respond to S1P in a fundamentally different manner than standard chemokines.

In response to S1P, cells initially display actin-dependent membrane ruffling similar to that observed in cells responding to CCL19, but S1P-treated cells rapidly transition into a series of contraction or squeezing events. *In vivo*, these contraction events may facilitate egress through lymphatic sinuses or splenic vasculature. Like WT T cells, DKO T cells undergo contraction events, but the DKO cells display frequent and repeated instances of membrane- cortex separation or "blebbing", which likely precludes a coordinated S1P-dependent egress response. Current work is aimed at defining both the mechanism of contraction as well as the dynamics of the plasma membrane, the actin cortex, and ERM proteins during S1P responses.

S1E. "Dissection of chromatin reader function in transcription using chimeric BET proteins"

Michael Werner, Hongxin Wang, Nicole Hamagami, Sarah Hsu, Aaron Stonestrom, Gerd Blobel

Chromatin reader proteins are a class of transcription cofactors that bind to chemically modified chromatin and are the target of therapeutic inhibition in dozens of clinical trials. In this study we dissect the function of the bromodomain and extra-terminal motif (BET) proteins Brd2, Brd3 and Brd4 using red cell development. Structure-function domain mapping of these highly conserved proteins reveals that the shared functionality of Brd2 and Brd3 can be mapped to a unique C- terminal protein-protein interaction domain, not the chromatin-binding domains. Protein interaction analysis implicates Brd2 in the function of RNA polymerase 2 associated factor complex (PAF) and casein kinase 2 complex (CK2), both of which have known roles in transcriptional pause-release and elongation. Ongoing studies examine the functional significance of the Brd2-PAF-CK2 interactions in transcription using rapid Brd2 depletion and CK2 inhibition.

S2A. "Allergic airway inflammation and airway hyperresponsiveness are independently controlled by diacylglycerol kinase"

Brenal K. Singh, Wen Lu, Amanda M. Schmidt Paustian, Moyar Q. Ge, Cynthia J. Koziol-White, Cameron H. Flayer, Sara S. Killingbeck, Nadan Wang, Xinzhong Dong, Matthew J. Riese, Deepak A. Deshpande, Reynold A. Panettieri, Jr., Angela Haczku, and Taku Kambayashi

Asthma is a chronic allergic inflammatory airway disease caused by aberrant immune responses to inhaled allergens, leading to airway obstruction caused by airway hyperresponsiveness (AHR) to contractile agonists. Here, we report that targeting diacylglycerol (DAG) kinase zeta (DGKζ), a negative regulator of DAG-mediated cell signaling, protects against allergic asthma by simultaneously blocking airway inflammation and AHR by independent mechanisms. Targeted deletion of DGKζ in T cells led to decreased type 2 inflammation with no attenuation of AHR. In contrast, loss of DGKζ in airway smooth muscle cells led to decreased AHR despite no changes in airway inflammation. Importantly, pharmacological inhibition of DGK diminished airway inflammation and AHR in mice, and also reduced bronchoconstriction of human airways. These data suggest that DGK is a novel therapeutic target for asthma and reveals that the inflammatory and AHR components of asthma are not as interdependent as generally believed.

S2B. "Role of the tissue barriers in the neuroimmune mechanisms of schizophrenia"

Alexis Crockett, Sean Ryan, Caroline Canning, Nickole Kanyuch, Richa Kapoor, Trini Ochoa, James Gesualdi, Stewart Anderson, Jorge Alvarez

Schizophrenia (SZ) presents as a relapsing remitting disease that correlate with upregulation of peripheral cytokines during psychotic episodes. While this implicates inflammation in the pathobiology of SZ, the mechanisms responsible for these effects remain unclear. Recent studies suggest that abnormalities in tissue barriers, including the blood-brain barrier (BBB) and the gastrointestinal barrier, might influence the manifestation of psychosis. We have investigated whether tissue barriers are intrinsically compromised in the context of the most common genetic risk factor for SZ, a hemizygous deletion of chromosome 22q11.2 (22q11DS). This disorder confers a 30-fold risk for psychosis and includes claudin- 5, a tight junction protein expressed in both the BBB and gastrointestinal barrier, in its deleted region. To investigate the role of the BBB in schizophrenia, we employed an innovative approach based on the differentiation of human 22q11.2+SZ induced pluripotent stem cells (iPSCs) into BBB endothelium (iBBB). We found that the schizophrenic iBBB exhibited impaired barrier integrity and compromised immune privilege properties, such that the expression of proinflammatory cell adhesion molecules were upregulated, while molecules associated with endothelial immunoquiescence were downregulated. These results were further substantiated in an *in vivo* rodent model of 22q11.2, which we also employed to investigate the gastrointestinal compartment. We have also observed impaired gastrointestinal barrier function in a rodent model of 22q11.2, including possible bacterial translocation, differential fecal Ig coating, and microbiome changes. Additionally, we found changes within the gastrointestinal immune compartment which suggest that the impaired gastrointestinal barrier function in 22q11.2 is associated with enhanced antigen presentation by myeloid cells and a resulting increase in inhibitory receptor expression on CD4+ T cells. These results indicate that SZ in the context of 22q11DS is associated with changes in tissue barrier functions that have immunological consequences, and suggest that these may interact to play an active role in neuropsychiatric disease.

S2C. "Lingo3 interacts with TFF2 to control mucosal integrity, Type 1 inflammation, and colitic tissue repair"

Kelly M Zullo, Yingbiao Ji, Rachel Cohen, Karl Herbine, Nicole Maloney, Li-Yin Hung, Michael Kohanski, Noam Cohen, De'Broski R. Herbert

Intestinal epithelia are constantly exposed to potentially damaging stimuli, which requires rapid repair to maintain tissue homeostasis and immunological quiescence. The reparative cytokine Trefoil factor 2 (TFF2) enforces lung and intestinal barrier integrity, but whether a TFF2 receptor exists is controversial. Herein, we demonstrate leucine rich repeat nogo interacting protein 3 (LINGO3) as a necessary transmembrane component for TFF2-mediated ERK signaling, proliferation of intestinal epithelia, and TFF2-mediated recovery of trans-epithelial resistance in scratch-wound assays. Human intestinal epithelia expressed LINGO3 and mice lacking LINGO3 had impaired intestinal barrier function, failed to recover from DSS-induced colitis, and showed both impaired crypt regeneration and LGR5 stem cell marker expression compared to WT counterparts. Lingo3$^{-/-}$ mice displayed increased paracellular permeability, and significant accumulation of mucosal CD4^{+} T_H1 cells expressing IFNg^{+} TNFa^{+} under steady-state conditions. Combined, these data reveal imply that a TFF2-LINGO3 ligand receptor axis regulates mucosal barrier integrity and GI inflammation.

S3A. "Characterizing the role of long noncoding RNAs in innate antiviral immune responses"

Megha G. Basavappa, Kanupriya Whig, David C. Schultz, Jorge Henao-Mejia, Sara Cherry

Emerging viruses, such as Zika virus (ZIKV) and chikungunya virus (CHIKV), pose a substantial public health threat often causing severe, symptomatic disease in endemic. Innate immunity is the first line of defense to combat these pathogens. While many of the proteins that drive innate immune signaling pathways have been described, less is known about the RNA-dependent regulatory mechanisms that modulate the expression and function of these proteins. Long noncoding RNAs (lncRNAs) are a recently identified class of regulatory RNAs. The contribution of lncRNAs to innate antiviral immunity is only beginning to be appreciated. To determine the role of lncRNAs in antiviral responses, we performed an unbiased, high-throughput, RNAi screen targeting 2200 lncRNAs in human brain microvascular endothelial cells (HBMEC) infected with either CHIKV or ZIKV. Viral infection was measured using immunofluorescence microscopy and automated image analysis. We identified over 100 lncRNAs that upon depletion, resulted in increased viral infection in the absence of cytotoxicity. Further exploration of these lncRNAs revealed that a subset of these lncRNAs are nuclear while others are cytoplasmic, suggesting distinct modes of action. Interestingly, the vast majority of lncRNAs that impact infection are virus-specific implying selectivity in host responses to these pathogens. Future mechanistic studies will define the molecular function of these lncRNAs in innate antiviral defense against these emerging viruses.

S3B. "Caspase-8 promotes c-Rel-dependent inflammatory cytokine expression and resistance against *Toxoplasma gondii*"

Alexandra A. DeLaney, Corbett Berry, Andrew Hart, David A. Christian, Elisabet Bjanes, Irina Udalova, Christopher A. Hunter, Igor E. Brodsky

Caspase-8 is a key integrator of cell survival or death decisions during infection and inflammation because it initiates cell-extrinsic apoptosis following engagement of TNF superfamily receptors or certain Toll-like receptors, as well as prevents activation of RIPK3-dependent programmed necrosis. Caspase-8 also has an important but poorly-defined role in regulating cell-intrinsic inflammatory gene expression. Notably, macrophages from mice deficient in RIPK3 and caspase-8 or its adaptor FADD, but not RIPK3 alone, have defects in expression of inflammatory cytokines or in inflammasome priming in response to bacterial infection or TLR stimulation. How caspase-8 regulates gene expression, and whether caspase-8-mediated gene regulation has physiological roles during infection remains unclear. Here we demonstrate that regulation of inflammatory gene expression involves both caspase-8 activity and scaffolding functions. Importantly, caspase-8 enzymatic activity was necessary for maximal expression of the inflammatory cytokines, *Il1b* and *Il12,* following TLR stimulation. Mechanistically, caspase activity was required for optimal IKK phosphorylation following multiple TLR stimuli, and furthermore, for robust nuclear translocation of the NF-kB family member c-Rel. Expression of c-Rel in *Ripk3*$^{-/-}$*Casp8*$^{-/-}$ macrophages restored their ability to synthesize pro-IL-1β and IL-12. Intriguingly, *Ripk3*$^{-/-}$*Casp8*$^{-/-}$ mice were severely compromised in their ability to resist *Toxoplasma gondii* infection, a protozoan parasite whose control requires IL-12. Importantly, addition of exogenous IL-12 was sufficient to rescued the survival of *Toxoplasma*-infected *Ripk3*$^{-/-}$*Casp8*$^{-/-}$ mice, thereby implicating caspase-8-dependent control of inflammatory gene expression in host defense against intracellular pathogens.

S3C. "Detection of the bacterial Type III Secretion System and Flagellin by the human NAIP/NLRC4 inflammasome

Valeria M. Reyes Ruiz, Sunny Shin

The innate immune response is critical for antibacterial defense. In turn, gram- negative bacteria, such as *Salmonella* Typhimurium, often employ type III secretion systems (T3SS) to inject effector proteins into the host to promote invasion and survival. However, these secretion systems also translocate structural components, such as the T3SS needle protein, T3SS inner rod protein, and flagellin, that are detected by cytosolic immune sensors termed NAIPs. The NAIPs trigger assembly of the inflammasome, a multi-protein complex that activates caspases to induce host cell death and IL-1 cytokine release. Unlike mice, which encode seven distinct NAIPs that each recognizes a different bacterial ligand, humans encode only one NAIP. Previous studies have shown that human NAIP detects both flagellin and the T3SS needle protein, and suggested that the ability to detect both ligands was achieved by multiple isoforms encoded by the single human *NAIP* gene. We have shown that human NAIP also senses the *Salmonella* Typhimurium T3SS inner rod protein PrgJ. In addition, our data show that the *Salmonella* Typhimurium SPI-2 T3SS inner rod protein, SsaI, which is required for intracellular bacterial replication, does not activate the inflammasome in human macrophages. Furthermore, we demonstrate that a single human NAIP isoform is capable of sensing the T3SS inner rod, needle, and flagellin. Our findings indicate that in contrast to murine NAIPs, promiscuous recognition of multiple bacterial ligands is conferred by a single human NAIP. Future experiments will determine the role of human NAIP in mediating cell-intrinsic responses against *Salmonella* infection.

S3D. "Trib1 controls antiviral immunity by restraining CD4 and CD8 T cell effector responses during chronic infection"

Kelly S. Rome, Sarah J. Stein, Makoto Kurachi, Ethan A. Mack, Sacha Uljon, Winona W. Wu, Stephen C. Blacklow, E. John Wherry, Martha S. Jordan, Warren S. Pear

During a chronic infection, the magnitude of the anti-viral T cell response is dictated by the balance between T cell effector (T_{EFF}) function and T cell exhaustion. While exhaustion has been extensively characterized, the regulation of T_{EFF} responses during chronic antigen exposure is less clear. We identified Tribbles Pseudokinase 1 (Trib1) as a central regulator of the T_{EFF} response to chronic infection. Trib1 expression is induced in activated T cells and in T cells from infected mice. T cell specific deletion of Trib1 enhanced T_{EFF} expansion and function and reduced viral burden in chronically infected mice. Additionally, Trib1-deficiency promoted both CD4 and CD8 effector responses. Consistent with this, we observed increased IL-2 production from Trib1- deficient CD4 T cells. Further, we observed enhanced T cell proliferation ex vivo in CD4 and CD8 T cells lacking Trib1, and demonstrate that Trib1 can restrain T_{EFF} responses via CD4 dependent and independent mechanisms. Combined, our data identify a critical role for Trib1 in regulating antiviral immunity, and provide important insight into restoring effector T cell function during chronic disease.

S4A. "The cellular identity of exhaustion: lessons from an *in vitro* model"

Jennifer E. Wu, Sasikanth Manne, Josephine R. Giles, Omar Khan, Ryan P. Staupe, E. John Wherry

T cell exhaustion is an acquired state of immune dysfunction that arises due to persistent antigenic stimulation in the context of chronic viral infections and cancer. Exhausted CD8 T cells are characterized by loss of effector function (such as cytokine production and cytotoxicity), decreased proliferative potential, increased expression of inhibitory receptors, and a unique transcriptional and epigenetic profile. This hypofunctionality ultimately results in diminished control and incomplete clearance of infection. Therapeutic reversal of T cell exhaustion via IR blockade is revolutionizing treatments of human cancers; however, these therapies are only capable of inducing transient cellular effects and are furthermore frequently associated with autoinflammatory adverse effects. A more thorough understanding of the biological underpinnings of exhaustion is needed to improve these therapies.

Our fundamental understanding of T cell exhaustion has predominantly come from studies in mice infected with the chronic strain of lymphocytic choriomeningitis virus (LCMV) known as Clone 13. Although powerful, this *in vivo* model has limitations. Because there are multiple determinants of exhaustion, the effects of individual pathways can be difficult to isolate. Furthermore, *in vivo* models generate low cellular yields which prohibit the use of various exploratory platforms. An *in vitro* model of exhaustion, however, would address both of these issues, allowing for the directed study of individual exhaustion-associated pathways in a scalable manner.

In our model, CD8 T cells are repeatedly stimulated *in vitro* with LCMV peptide (via presentation by dendritic cells) in the presence of low-dose IL-2. This method of stimulation is sufficient to induce three main modules of the exhaustion phenotype: 1) high expression of inhibitory receptors, 2) certain aspects of the exhaustion- associated transcription factor signature, and 3) decreased effector cytokine production. This *in vitro* modeling of CD8 T cell exhaustion is also sufficient to partially recreate the transcriptional and epigenetic signatures of bona fide *in vivo* exhausted T cells, although certain key elements (for example, downstream programs associated with the transcription factors Tox and Tcf7) are absent from the *in vitro* signatures. However, these *in vitro* stimulation conditions are sufficient to commit these cells to a exhaustion lineage: when transferred into LCMV-infected mice, they maintain high inhibitory receptor expression *in vivo* and preferentially persist and proliferate in chronic (as opposed to acute) infection, as has been previously demonstrated for bona fide exhausted CD8 T cells. Thus *in vitro* approaches can be a useful tool to model the effects of various external signals on discrete aspects of the exhaustion phenotype and cellular identity.

S4B. "Role of Retinoic Acid on Mononuclear Phagocytes in the Sarcoma Microenvironment"

Samir Devalaraja, Jerrick To, Ian Folkert, Minghong Li, Yuma Tada, Malay Haldar

Soft tissue sarcoma (STS) is a rare but heterogeneous collection of fatal malignancies that arise from mesenchymal tissue such as fat, muscle, cartilage, etc. Recent efforts to utilize immunotherapies such as immune checkpoint blockade in STS have demonstrated efficacy only in a small percentage of patients, underscoring the importance of elucidating additional immune evasion mechanisms.

It is now understood that immunosuppressive tumor associated macrophages (TAMs) are abundant in the solid tumor microenvironment (TME) and pose a major barrier to successful anti-tumor immunity. On the other hand, immunostimulatory dendritic cells (DCs) are a developmentally related cell type that are rare in the TME but necessary to generate effective anti-tumor T cell responses. Though both TAMs and DCs can arise from monocytes, why the majority of monocytes in the TME preferentially differentiate into TAMs and not DCs is unknown.

We have uncovered that sarcoma cells produce high levels of retinoic acid (RA), which promotes the generation of immunosuppressive TAMs but inhibits DC differentiation from monocytes. Notably, sarcomas that were genetically modified to produce limited amounts of RA demonstrated enhanced T cell dependent anti-tumor immunity. Current work explores the regulation of RA production in the TME, cellular mechanisms by which RA inhibits anti-tumor immune responses and transcriptional mechanisms by which RA influences monocyte differentiation.

S4C. "Intratumoral Immune Activation Informs Rational CAR T Cell Design"

Lexus R. Johnson, Carl H. June, Andy J Minn

Inflammatory cues drive immune activation as well cancer cell-intrinsic resistance programs in the tumor microenvironment. The balance between these genetic signatures is an important determinant in the response immune checkpoint blockade (ICB), and thus the identification of cues that favor immune activation may provide novel methods for improving immune responses against cancer. We have recently identified the highly structured RNA RN7SL1 as capable of stimulating immune response genes in tumor cells following secretion by neighboring fibroblasts. Thus, unshielded RN7SL1 in the tumor microenvironment may represent a naturally occurring DAMP with the ability to act on both tumor and immune populations. We show that RN7SL1 is stimulatory to primary human DCs, and that increasing the amount of unshielded RN7SL1 present in the tumor microenvironment (TME) increases the frequency of infiltrating DCs and enhances T cell activation. This correlates with improved responses to ICB in multiple contexts. Interestingly, tumors that overexpress RN7SL1 grow more robustly in immune-deficient mice than control tumors, highlighting an opposing role for DAMP signaling in tumor resistance. Given these dichotomous roles, our work identifies the recruitment and anti-inflammatory polarization of myeloid cells in the tumor microenvironment as a potential regulator of outcomes following the activation of PRR signaling by RN7SL1, wherein the restraint of T cell activation allows for robust tumor growth, while absence of these cells favors immune activation and tumor rejection. In order to deliver this stimulatory RNA in a therapeutic setting, we have designed a T cell-based system for delivering RN7SL1 directly to the tumor microenvironment. Following treatment with RN7SL1-producing CAR T cells, we observed enhanced recruitment of DCs and T cell activation. When combined with ICB therapy, delivery of RN7SL1 via CAR T cell synergizes with treatment and delivers robust improvement of survival outcomes. Thus, the delivery of stimulatory RNA in this fashion may represent an opportunity to leverage beneficial aspects of both CAR T and ICB therapies in solid tumors that are currently refractory to available treatments.

P1. "Investigating the MHC Class II-restricted processing landscape of HIV-1 antigens"

Mary Margaret Addison, Laurence Eisenlohr

Robust HIV-specific CD4 T cell (T_{CD4}) responses are associated with decreased viral load and a slower progression to AIDS, and should therefore be considered in HIV vaccine development.

T_{CD4} are activated by antigen-derived peptides displayed in complex with MHCII on the surface of antigen presenting cells (APC). By convention, internalized antigens are processed and loaded onto MHCII in late endosomal compartments. However, alternative pathways, such as endogenous processing, have been described. This occurs when viral antigens produced within infected APCs are proteolyzed and loaded onto MHCII by a network of intracellular pathways.

The relative contributions of these pathways to the HIV-specific T_{CD4} response are unknown. Additionally, our understanding of the cell types that act as APCs during HIV infection is incomplete, as T_{CD4}, which express MHCII upon activation and are HIV host cells, might act in this capacity. *In vitro* assays have revealed significant heterogeneity in the ways in which primary DCs and macrophages process HIV proteins, while activated T_{CD4} are able to present antigen derived from a live HIV infection via endogenous processing.

P2. "Skewed T cell distribution in pancreatic cancer patients"

Cécile Alanio, Katelyn T. Byrne, Takuya Ohtani, Josephine Giles, Bertram Bengsch, Sarah Henrickson, Weng Nan Ping, Janáe A. Ritz-Romeo, Mark O'Hara, J. Joseph Melenhorst, Simon Lacey, Regina M. Young, Robert H. Vonderheide, Carl H. June, E. John Wherry

To understand potential immune system alterations in newly diagnosed, untreated, pancreatic cancer patients and provide a foundation for immunotherapy, we profiled PBMC from pancreatic ductal adenocarcinoma (PDA) patients and age matched healthy controls using high dimensional CyTOF analysis. We developed two immune profiling panels: a *broad* profiling panel that includes 45 phenotypic markers that together permit the identification and enumeration of the main innate and adaptive immune cell subsets in humans, and a *deep* profiling panel that includes 45 features focusing on T cell phenotype and biology. We report a 2-fold increase in monocytes, more regulatory T cells, and more plasmocytes in circulation in pancreatic cancer patients compared to age-matched controls, as well as a bias towards cytokine-producing NK cells. Using high dimensional approaches, we observe skewed T cell differentiation in pancreatic cancer patients, with CD8 T cells biased towards more CD45RA- positive CD27-positive CCR7-positive CD95-positive CD49d-positive stem cell memory cell ($P=3x10^{-4}$), more CD45RA-negative CD27-positive CCR7-negative effector memory cells (P=0.002) and less CD45RA-positive CD27-negative CCR7-negative late effector memory cells (P=0.01) than age-matched controls. We further examined alterations of T cell differentiation in CD8 T cell compartment in human spleens, and report increased proportions of late

effector memory T cells in pancreatic cancer patients as compared to age- matched controls. These results reveal a trafficking defect of late memory T cells in pancreatic cancer patients. We are now investigating the mechanisms underlying these observations, as well as their impact on T cell immunity of the patients. Our goal is to understand the nature of the skewing and how any changes in baseline immune health of the T cell compartment related to disease progression and/or response to therapy. These studies should provide a foundation for improving therapy in pancreatic cancer patients.

P3. "Original antigenic sin priming of hemagglutinin stalk antibodies"

Claudia Arevalo, Scott Hensley

Initial childhood influenza virus encounters can leave lifelong immunological 'imprints' that affect immune responses against subsequent infections with antigenically distinct viral strains. Recent studies suggest that individuals infected with 'group 1' or 'group 2' influenza A early in life have protection against new pandemic strains of the same group. This protection is suggested to occur at the level of stalk antibodies targeting conserved regions among viruses within the same group or subtype. However, much less is known about how early childhood infections with one subtype affect responses to a second and different subtype. To address this, we infected ferrets sequentially with a group 1 (H1N1) followed by a group 2 (H3N2) virus and vice versa. Antibody levels elicited against H3N2 were similar in animals with and without prior H1N1 exposure, and the same applied to the reverse infection order. Surprisingly, specificity differed in animals with a prior infection, where the second infection boosted a stalk response to the virus of the first infection. These studies might explain age distribution of susceptibility to different pandemic strains.

P4. "Determining the role of human GBP1 in inflammasome activation during Legionella pneumophila infection"

Antonia Bass, Sunny Shin

Host recognition of intracellular bacterial pathogens results in the formation of a multiprotein complex termed the inflammasome, which leads to proinflammatory cytokine secretion and pyroptosis, an inflammatory form of cell death. Two forms of the inflammasome are the caspase-1 dependent canonical inflammasome and the caspase-1 independent noncanonical inflammasome. Both inflammasomes are promoted by interferon receptor signaling. A family of interferon-inducible GTPases known as guanylate binding proteins (GBPs) are a family of host cell factors that promote inflammasome response to a variety of bacteria in mice. Specifically,
interferon-gamma (IFN-γ) is a cytokine that is a potent inducer of GBPs in mouse macrophages. Mice possess 11 GBPs on chromosomes 3 and 5, whereas humans have 7 GBPs on chromosome 1. GBP functions in mice include bacteriolysis of cytosolic bacteria and rupture of pathogen-containing vacuoles in order to release pathogen-derived products into the cytosol, resulting in host recognition and inflammasome activation. Whether IFN-γ promotes inflammasome activation and upregulates GBPs in human macrophages is unknown. In this study, we use *Legionella pneumophila*, an intracellular gram-negative bacteria, to study innate immune response. We hypothesize that human GBPs play a role similar to mouse GBPs through membrane rupture of the *Legionella*-containing vacuole to promote inflammasome response. To do this, we conducted IFN-γ priming experiments to look at whether IFN-γ promotes inflammasome response and upregulates GBP expression in primary human macrophages. We show that IFN-γ

upregulates inflammasome response to *Legionella* and induces human GBP expression. Additionally, we employed siRNA-mediated knockdown of GBPs to determine whether they play a role in inflammasome response to *Legionella*. We found that human GBP1 promotes cell death and IL-18 cytokine secretion. Together, our results indicate that IFN-γ-induced human GBP1 is a key factor in inflammasome activation during *Legionella* infection. This study elucidates aspects of human innate immune response to gram-negative bacterial pathogens.

P5. "Mitochondrial protein CARD19 regulates a cell death checkpoint downstream of caspase activation and Gasdermin D and Gasdermin E/DFNA5 cleavage"

Elisabet Bjanes, Kariana Rios, Alexandra DeLaney, Eric Rodriguez Lopez, Baofeng Hu, Naomi H. Philip, Dorothy Tovar, Brian C. Schaefer, Igor E. Brodsky

Distinct forms of cell death play a critical role in tissue homeostasis and inflammatory responses to cellular stresses and microbial infections. During pyroptosis, Gasdermin D (GSDMD) is cleaved to enable IL-1 cytokine release and terminal cell lysis. Similarly, during apoptosis, cleaved Gasdermin E forms plasma membrane pores that mediate cytokine release and lysis. Nevertheless, certain stimuli or cell types induce IL-1 cytokine release via formation of plasma membrane GSDMD pores without inducing lysis, suggesting that these two responses can be decoupled. How the final steps of cell death might be regulated downstream of GSDMD/E pore formation is unknown. Here we demonstrate that the mitochondrial CARD-protein, CARD19, regulates lysis downstream of multiple caspase-dependent death pathways. Despite marked protection from cell death, *Card19*$^{-/-}$ macrophages had no defect in caspase activation, IL-1 cytokine secretion, or GSDMD/E cleavage. Notably, CARD19-deficiency was associated with reduced levels of cleaved GSDMD/E at the plasma membrane. These findings genetically uncouple for the first time, cell death from caspase processing, IL-1 release, and GSDMD/E cleavage and demonstrate that CARD19 regulates a cell death checkpoint downstream of caspase-dependent GSDMD/E cleavage.

P6. "Regulation of CD28 costimulation on signal-induced alternative splicing events in human CD4^{+} T cells"

Davia Blake, Kristen Lynch

Alternative splicing consists of exons that are either selectively included or excluded from the final mRNA transcript. Previous studies recognized that approximately 10% of alternatively spliced genes undergo signal-induced changes in isoform abundance upon T cell activation. Overall, the field lacks general knowledge of how splicing changes impact T cell function and how splicing changes are regulated. Preliminary data suggests that the induction of alternative splicing changes is differentially regulated by CD3 and CD28 stimuli. For example, some alternative splicing events need only CD3 stimulation for the induction of significant alternative splicing changes, while others require both CD3 and CD28 stimulation. Our current work seeks to fully classify alternative splicing events that are regulated dependently or independently of CD28 co-stimulation, and to ask the question of whether splicing plays a functional role in T cell activation. This work will uncover the regulatory heterogeneity of induced alternative splicing changes and will allow us to elucidate mechanisms of how signaling events downstream of T cell activation mechanistically control induced alternative splicing changes.

P7. "Modulation of T-cell activation by dendritic cell stiffness"

Blumenthal D, Burkhardt JK

Dendritic cells (DCs) responding to inflammatory stimuli undergo a process of maturation, through which they become highly effective APCs specialized for T-cell priming. Activation of T-cells takes place at a specialized cell- cell contact site termed the immunological synapse (IS), where multiple receptor-ligand interactions work in concert to direct the T-cell response. Signaling events at the IS depend on forces exerted on the DC by the T-cell actin cytoskeleton. We hypothesize that maturation-associated changes in the DC cytoskeleton alter the biophysical properties of the DC cortex, thereby serving as a platform for enhanced mechanotransduction in interacting T-cells. Using atomic force microscopy, we show that upon maturation, DC stiffness increases two to three fold via an actin- dependent process. Pharmacological studies and analysis of DCs from KO mice reveal that this process depends on actin polymerization downstream of both Arp2/3 complex and formins. Using acrylamide hydrogels coated with stimulatory ligands, we find that T-cell activation is enhanced by increases in stiffness similar to those observed during DC maturation. Dose-response studies reveal that increased substrate stiffness reduces the agonist dose needed to initiate T-cell activation, indicating that mechanical cues function as co-stimulatory signals. Stiffness sensitivity is conserved in $CD4^+$ and $CD8^+$ T-cells, and affects both priming and effector functions. Strikingly, we show blood-derived T-cells lack stiffness sensitivity, suggesting that mechanosensing can be switched off. Finally, by engineering DCs with altered stiffness, we show that stiffness of mature DCs directly correlates with their ability to prime *ex-vivo* T-cells. Taken together, these data reveal a novel mechanism for regulation of T-cell priming by DCs and identify cortical stiffness as an unexplored control point for T-cell priming.

P8. "MicroRNA profiling reveals miR-29a as a key regulator of CD8 T cell responses to viral infection"

Zhangying Cai, Erietta Stelekati, Sasikanth Manne, Kito Nzingha, Zeyu Chen, Viktoriya Ekshyyan, Christina Niavi, Shin Ngiow, Mohammed-Alkhatim Ali, Makoto Kurachi, and E. John Wherry

Persistent antigen exposure during chronic infection or cancer leads to T cell exhaustion. Multiple transcription factors have been shown to regulate the development of exhausted T cells, while the translational regulatory machinery remains poorly understood. With a specific interest in microRNAs, we compared microRNA profiling data at different infection stages and identified microRNA-29a as a key regulator of T cell differentiation. Overexpression of microRNA-29a enhanced CD8 T cell expansion, increased their effector function, promoted a memory phenotype and attenuated exhaustion upon chronic infection. Adoptive transfer of microRNA-29a-overexpressing transduced CD8 T cells reduced chronic LCMV viral loads compared to control transduced cells. Further, microRNA-29a-overexpressing transduced CD8 T cells exposed to chronic infection expanded more than control transduced cells in response to a secondary antigen re-challenge. Thus, microRNA-29a inhibits T cell exhaustion by promoting memory CD8 T cell differentiation during chronic infection and provides a potential target for microRNA therapeutics.

P9. "Role of Thymic Stromal Lymphopoietin (TSLP) in Mediating Protection from High Fat Diet (HFD) Induced Diabetes"

Ruth Choa, Shogo Wada, Jun Tohyama, Patrick Lundgren, Brenal Singh, Yukinori Tanaka, Ruth-Anne Langan, Zoltan Arany, and Taku Kambayashi

Inflammatory cues drive immune activation as well cancer cell-intrinsic resistance programs in the tumor microenvironment. The balance between these genetic signatures is an important determinant in the response immune checkpoint blockade (ICB), and thus the identification of cues that favor immune activation may provide novel methods for improving immune responses against cancer. We have recently identified the highly structured RNA RN7SL1 as capable of stimulating immune response genes in tumor cells following secretion by neighboring fibroblasts. Thus, unshielded RN7SL1 in the tumor microenvironment may represent a naturally occurring DAMP with the ability to act on both tumor and immune populations. We show that RN7SL1 is stimulatory to primary human DCs, and that increasing the amount of unshielded RN7SL1 present in the tumor microenvironment (TME) increases the frequency of infiltrating DCs and enhances T cell activation. This correlates with improved responses to ICB in multiple contexts. Interestingly, tumors that overexpress RN7SL1 grow more robustly in immune-deficient mice than control tumors, highlighting an opposing role for DAMP signaling in tumor resistance. Given these dichotomous roles, our work identifies the recruitment and anti

P10. "IL-33 Amplifies the Inflammatory Response to T. gondii"

Joseph Clark, Christopher Hunter

Mice deficient in IL-33 or its receptor ST2 are susceptible to infection with the protozoan parasite Toxoplasma gondii, but how IL-33 promotes resistance to this infection is unclear. Previous reports have attributed this susceptibility to an excessive type 1 response and lethal immune pathology in the central nervous system (CNS). In recent years, a direct role for IL-33/ST2 in enhancing type 1 responses has been appreciated. In the current studies, we focused on the role of IL-33 in the early innate response to T. gondii infection by using $Rag^{-/-}$ mice. In vitro, we found that IL-33 stimulation amplified IL-12-dependent NK cell production of IFN-γ and GM-CSF. In vivo, treatment of Toxoplasma-infected $Rag^{-/-}$ mice led to the recruitment and expansion of $CCR2^{+}$ monocytic cells at the site of infection in an IL-12 and IFNg-dependent manner. This treatment led to upregulation of iNOS in tissues and a marked reduction in parasite burden. These results suggest that IL-33 enhances the inflammatory response to T. gondii and consequently control of this parasitic infection.

P11. "Dissecting the tissue-specific mechanisms that support the maintenance of intestinal regulatory T cells"

Elisa Cruz-Morales, Andrew Hart, and Terri M. Laufer

Regulatory T cells (Tregs) in the intestinal mucosa play an important role in the maintenance of tolerance toward food antigens and commensals. Understanding the mechanisms for localization and maintenance of Tregs in the intestinal niche will help to identify novel immunotherapies for inflammatory intestinal diseases.

Similar to other CD4 T cells, the clonal expansion of Tregs seems to require TCR-dependent activation by MHCII-peptide complexes. However, our laboratory previously used the K14 transgenic mouse lacking peripheral TCR-MHCII interactions to show that the small intestine lamina propria (siLP) contains a niche for Tregs that can be filled and maintained independently of such antigen specific signals. We are now interested in understanding the mechanisms that sustain these MHCII-independent Treg in the intestinal niche.

Tregs can be phenotypically and functionally divided into central and effector Tregs. Central Tregs preferentially migrate from thymus to populate secondary lymphoid tissues. Effector Tregs reside mainly in non-lymphoid tissues and acquire a tissue-specific phenotype. Although it has been proposed that antigen-specific signals mediate the differentiation of effector Tregs, we find that siLP Tregs acquire an effector phenotype and transcriptional signature independently of local MHCII; rather, MHCII-independent costimulatory signals are important to maintain Treg proliferation in the siLP. Additionally we find that in MHCII-deprived intestinal niche, Tregs are disproportionately localized in organized lymphoid structures known as isolated lymphoid follicles (ILFs) where they are in proximity to B cells and dendritic cells.

Our results suggest that MHCII-independent effector Tregs may be generated and maintained via distinct costimulatory signals and highlight the importance of the second costimulatory signal during the antigen presenting cell (APC)-Tregs interaction.

P12. "Cul4b regulates proliferation and effector function of T cells"

Asif Dar, Joseph Dybas, Keisuke Sawada, Emily Moser, Paula Oliver

The capacity for T cells to become activated and rapidly expand during pathogen invasion is a core function of the adaptive immune system and this aids pathogen clearance. When T cells fail to expand, there is a collapse of adaptive immunity and pathogen clearance is impaired. Defining factors that regulate T cell expansion could allow development of therapeutics designed to regulate the numbers of antigen-specific T cells. Following activation, T cells employ both transcriptional and post-translational mechanisms to regulate the levels of proteins that sustain the cells metabolic needs and allow clonal expansion. While new sequencing technologies have fundamentally enriched our understanding of transcriptional changes that support T cell activation, far less is known about post-translational changes, such as ubiquitylation. Here we show that these two processes are inextricably linked. Using a screen we developed to assess cullin E3 ligase activity following T cell activation, we identified Cul4b, a cullin known only for its role in promoting tumor growth. Using CRISPR, we then generated mice lacking Cul4b specifically in T cells. These mice revealed that Cul4b promotes the expansion, survival, and effector function of both CD4+ and CD8+ T cells following activation. We then employed our newly developed proteomics-based substrate screen and identified several potential substrates, including two linker histones (H1.1 and H1.2) that regulate core histone methylation and chromatin accessibility. Additionally, we found that H1.2 is physically associated with Cul4b in primary T cells. Our data support that Cul4b ubiquitylates specific linker histones such as H1.2 to modify gene accessibility and promote the expansion and function of antigen-specific T cells and adaptive immune responses. This information will have significant implications as how T cells expand during pathogen invasion to initiate protective immune responses.

P13. "Dendritic cells undergo apoptosis in response to bacterial blockade of host protein synthesis to restrict *Legionella pneumophila* infection"

Jessica Doerner, Jenna Zhang, Igor Brodsky, Sunny Shin

Early responses mounted by innate immune cells are essential for host defense against bacterial pathogens. Recognition of pathogen effector molecules by the host helps to initiate this response. Macrophages and dendritic cells (DCs) are important early responders, due to their phagocytic nature and anti-microbial cytokine responses. The gram-negative bacteria *Legionella pneumophila*, which causes a severe pneumonia-like disease, uses a type IV secretion system (T4SS) to inject effector proteins that manipulate host cell processes and enable *Legionella* to replicate within alveolar macrophages. Conversely, upon infection, DCs undergo rapid apoptosis in response to T4SS activity. Here, we sought to define the T4SS-translocated bacterial components that trigger apoptosis in DCs. We find a critical role for *Legionella* effector proteins that block host protein translation in inducing caspase cleavage and cell death in DCs. Thus, our data suggest that DC apoptosis is an effector-triggered immune response to bacterial blockade of host translation that enables cell-intrinsic restriction of bacterial replication in DCs.

P14. "Using a transgenic gastrointestinal nematode to study the differentiation, function and maintenance of antigen-specific type 2 (Th2) CD4+ T cells"

Bonnie Douglas, Yun Wei, Xinshe Li, Tom Nolan, James Lok, De'Broski Herbert

Th2 cells regulate helminth immunity and allergic inflammation, but Th2 cell activation and differentiation, migration, and maintenance are poorly understood. To study antigen-specific Th2 cells in helminth infection, we engineered a transgenic gastrointestinal (GI) nematode, *Strongyloides ratti*. This line, named *Hulk*, expresses immunodominant T cell epitope 2W1S as a fusion protein with green fluorescent protein (GFP) and FLAG in its body wall. *Hulk* has expressed GFP and FLAG for 7 generations in all life stages. Preliminary data show that *Hulk* can be visualized in live mouse skin using multiphoton microscopy, and ongoing imaging work focuses on CD11c+ populations potentially responsible for antigen uptake early in infection. Additionally, *Hulk* infection expands 2W1S:I-Ab+ CD4+ T cells in the lung at 7 and 14 days post-infection, but to a lesser extent than 2W1S peptide immunization. To enhance 2W1S-specific CD4+ T cell expansion, we are developing a new line of *S. ratti* from which 2 copies of 2W1S will be secreted. Our work may offer new insights into Th2 immunobiology while informing development of vaccines and therapies targeting Th2 cells.

P15. "The role of the E3 ubiquitin ligase Itch in CD4 T cell expansion"

Natania Field, Joseph Dybas, Emily Moser, Omar Elbulok, Paula Oliver

In order to mount a robust anti-pathogen response, effector CD4 T cells must undergo rapid proliferation. However, if T cell expansion is poorly controlled, excess inflammation can occur, damaging the host. One activation-induced change that allows T cells to proliferate rapidly is the upregulation of glycolytic activity. T cells can also regulate their expansion through metabolic-independent processes, such as cell cycle progression. Regulation of both metabolic dependent and independent processes is essential to maintaining T cell homeostasis.

One important regulator of CD4 T cell proliferation is the E3 ubiquitin ligase Itch. While the role of Itch in cytokine production has been extensively studied, it is unknown how Itch regulates T cell proliferation, and whether this activity of Itch affects T cell pathogenicity. We have found that Itch deficient T cells proliferate more *in vitro* and *in vivo* independently of cytokine production, correlating with increased capacity to cause disease. To determine how Itch controls proliferation, we conducted an analysis of the whole cell proteome of Itch deficient CD4 T cells compared to Itch sufficient controls. Pathway analysis revealed dysregulation of metabolic and cell cycle activities. On a functional level, Itch did not affect T cell glycolytic capacity nor mitochondrial respiration, but Itch deficient T cells had increased entry into S phase that could not be explained by changes in activation status. In order to identify the specific substrates of Itch that regulate cell cycle, we plan to compare the proteomics data to transcriptomics data to determine which proteins are regulated post-translationally. We will then test whether these proteins are differentially degraded and ubiquitinated in Itch deficient cells, which would imply that they are Itch substrates. This project may reveal new mechanisms that limit T cell expansion.

P16. "ECTV-encoded protein B22 restricts CD4+ T cell activation"

Katherine Forsyth, Laurence Eisenlohr

Orthopoxviruses encode many immunomodulatory proteins that profoundly interfere with various aspects of the immune system. Ectromelia (ECTV) is the orthopoxvirus that naturally infects mice, affording the opportunity to study viral-host interactions that have co-evolved. Importantly, disease progression closely mimics that of monkeypox and smallpox in humans. CD4+ T cells are crucial for the control of ECTV, functioning to both directly kill infected cells and help B and CD8+ T cell activation. Here we show that ECTV directly counteracts CD4+ T cell activation through inhibition of MHC-II mediated peptide presentation by the infected cell. A promising candidate protein for this inhibition is B22, which localizes to the plasma membrane, has homologs in virulent but not vaccine strains and, indeed, contributes substantially to ECTV virulence. We show that B22 is both necessary and sufficient to restrict CD4+ T cell activation, with experiments ongoing to elucidate the specific mechanism of inhibition, which is not attributable to down-regulation of surface MHC-II nor release of soluble factors.

P17. "Assessing the basis of chromatin opening by hematopoietic pioneer factors"

Megan Frederick, Kenneth Zaret

Coordinated expression of PU.1 with myeloid TFs C/EBPα or C/EBPβ is required to give rise to macrophages and ectopic expression of PU.1 and C/EBPα/β converts fibroblasts to the macrophage lineage. Genomic experiments have demonstrated that both PU.1 and C/EBPα/β target closed chromatin resulting in nucleosome depletion and de novo accessibility of chromatin at macrophage-specific cis-regulatory elements. However, it remains unclear if PU.1, C/EBPα and C/EBPβ have the intrinsic ability to interact with closed chromatin and initiate accessibility. I hypothesize that PU.1, C/EBPα and C/EBPβ function as pioneer factors by directly binding silent chromatin to initiate chromatin opening. To test this, I have assembled nucleosome arrays containing a central nucleosome capable of specific binding by the aforementioned TFs. Using arrays compacted with linker histone, we reveal that PU.1 and C/EBPα appear to possess the

intrinsic ability to open closed chromatin. Future experimentation will test the molecular features that endow PU.1 and C/EBPα with the intrinsic ability to modulate chromatin structure.

P18. "mTORC1 signaling activates a proactive UPR in early plasma cell differentiation independent of XBP1 splicing"

Brian Gaudette, David Allman

The generation of antibody-secreting plasma cells (PCs) from mature, naïve B cells requires a shift in transcriptional programs leading to striking changes in cellular morphology and physiology. It is thought that the lynchpin of this process is initiation of the unfolded protein response (UPR) through activation of the XBP1 transcription factor downstream of Blimp1-dependent increases in immunoglobulin protein translation. The paradox of a purely reactive, endoplasmic reticulum (ER)-stress-dependent UPR in PC differentiation is the observed lack of PERK-dependent translation inhibition, which is intrinsic to the canonical UPR. By comparing the transcriptional and protein-level activation of mTORC1 and the UPR in two subsets of naïve B cells with different kinetics of PC differentiation, follicular and marginal zone (MZ) B cells, we are able to differentiate between mTORC1-dependent and ER stress-dependent activation of canonical UPR targets. Our results indicate that MZ B cells, which more rapidly differentiate to PCs, have increased mTORC1 activity at baseline, which is accompanied by increased expression of ER protein folding machinery in the absence of XBP1 activity. Furthermore, we demonstrate an mTORC1-dependent increase in expression of canonical UPR targets prior to Blimp1 induction and independent of XBP1 activity in naïve B cells under PC-inductive conditions. Thus, activation of the mTORC1 pathway in early PC differentiation primes the ER to prepare for rapid increase in protein production in the absence of canonical ER stress.

P19. "The blood meningeal barrier orchestrates the development of neuroinflammatory responses"

James Gesualdi, Miles Miller, Hania Kebir, Lara Cheslow, Guadalupe Ceja, Daniel Beiting, Alexandre Prat, Jorge Iván Alvarez

The central nervous system (CNS) barriers, namely the blood-brain barrier (BBB) and the blood meningeal barrier (BMB), regulate the movement of leukocytes and inflammatory mediators from peripheral blood into the CNS. Growing evidence indicates that interactions between leukocytes and the BMB are crucial in the formation of encephalitogenic CNS lesions. Thus, our group aims to characterize the mechanisms regulating immunological outcomes within the meningeal compartment. Leveraging a human in vitro model of the CNS barriers, we found the BMB displayed a more robust upregulation of pathways related to cell-cell adhesion, antigen processing and presentation, and cytokine and chemokine activity as compared to the BBB. As a result, CD4 T cells became more inflammatory upon migration across the BMB relative to the BBB, and B cells were more likely to migrate across, adhere to, and form aggregates upon contact with BMB endothelia. This penchant for the BMB is recapitulated by the distribution of B cells observed during both multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE), in which the meningeal compartment has been shown to support robust infiltration by encephalitogenic T and B cells to the point of

forming lymphoid aggregates resembling tertiary lymphoid organs. Further, the presence of these structures is correlated with a more severe disease course and exasperated neuropathology. This has lead us to reason that the BMB endothelium is a driver of immunopathology. We have shown that during neuroinflammation local vascular endothelia upregulate multiple cell-cell adhesion molecules, facilitating infiltration and associated pathology. As these molecules are known targets of NFκB signaling, we hypothesized that the absence of this pathway within the BMB would compromise meningeal inflammation. Indeed, mice bearing a conditional knockout of the IKK kinase complex in vascular endothelium displayed a significant reduction in clinical outcomes upon EAE induction. This phenotype was marked by an absence of TLO-like structures and submeningeal neuropathology, both of which are predominant in wild type littermate controls. Altogether, our findings indicate that the BMB niche is specifically conducive to the promotion of pathological inflammation observed during MS and EAE.

P20. "Effect of Natural Genetic Variation on the Chromatin Landscape During T Cell Development"

Naomi Goldman, Maria Fasolino, and Golnaz Vahedi

During T cell maturation in the thymus, progenitors commit to the T cell lineage by undergoing sequential cell fate decisions that are associated with specific changes in gene expression. These cell fate-specific gene expression programs are specified in part by alterations in chromatin accessibility via lineage-determining transcription factors (TFs). Work in our lab has recently identified that the transcription factor TCF-1— integral for normal thymic development—targets and is essential for the opening of repressed chromatin in T cells. In other cell-types, LDTFs are known to collaborate to organize a fully active enhancer. However, the nature of TCF-1's collaborative interactions with other TFs in selecting enhancers and how they work to regulate genes during T cell development is unknown. To address these questions, we utilized the natural genetic variation between two strains of mice (C57BL/6J (BL6) vs NOD/ShiLtJ (NOD) with 5.8 million single nucleotide polymorphisms (SNPs)) as an *in vivo* mutagenesis screen to determine the effects of SNPs on TF binding and collaboration, chromatin accessibility, genome organization, and gene expression in developing T cells.

P21. "The DLL1/Notch2 signaling axis regulates the maintenance of Marginal Zone B cells"

Daniela Gómez Atria, Brian Gaudette, Eric Perkey, Bhaskar Srivastava, Chris Siebel, David Allman, Ivan Maillard

Current models postulate that Notch2 regulates a binary cell fate decision that specifies Marginal Zone B (MZB) vs. follicular B (FOB) cell fates. In contrast, we hypothesize that Notch2 signaling mediated by stromal Delta-like1 ligands controls the maintenance of MZB cells and their strategic positioning in specialized areas of the spleen. To test this hypothesis, we administered antibodies that acutely and specifically block either Notch2 or its ligand DLL1 in mice. Numbers of FOB cells remained stable, but the relative abundance of MZB cells decreased rapidly within 24-48 hours after antibody treatment. To investigate Notch-regulated pathways, RNA-Seq analysis was performed in sorted FOB and MZB cells at 0, 12, 24 and 48 hours after blockade. Notch2 inhibition led to progressive profound changes in the MZB transcriptome, but to only modest changes in FOB cells. Early after blockade (12h), we identified downregulated expression of canonical transcriptional Notch targets and dysregulated expression of chemokine and S1P receptor

genes on MZB. At later time points, GSEA identified an impact of Notch inhibition on apoptotic pathways, the MYC regulome, mTORC1 signaling and the UPR. Moreover, we found that highly purified FOB cells transferred into RAG2-deficient lymphopenic hosts differentiated *via* a Notch-dependent process into MZB-like cells. Our data suggest that FOB cells are not terminally differentiated and that MZB cells rely on continuous Notch signals to maintain their metabolic state, functional readiness and persistence in the marginal zone.

P22. "Epigenetic regulation of follicular CD4 T helper cell function and differentiation in lupus"

Andrew Hart, Terri Laufer

Lupus is a complex autoimmune disease characterized by the presence of high affinity, class switched autoantibodies. Many genetic polymorphisms have been shown to associate with disease susceptibility. Of these, very few reside in gene-coding regions and mechanistically explain disease onset. How genetic susceptibility may lead to cellular dysfunction is not straightforward. Additionally, multiple aberrant B cell and T cell functions contribute to lupus development and progression and the relative effect and contributions of different cellular populations is still under investigation. Follicular CD4 helper T cells (Tfh), primarily known for mediating B cell maturation during germinal center reactions, are of particular interest for their potential to influence autoantibody development and subsequent disease. This study aims to understand how epigenetic alterations in lupus-derived Tfh cells influence their altered frequency and functions leading to disease.

P23. "Converting PDAC from "cold" to "hot""

Austin Huffman, Robert Vonderheide

It is increasingly clear that tumors with pre-existing T-cell infiltration – immunologically "hot" – are much more likely to respond to immune checkpoint blockade (ICB) therapy than tumors that are T-cell sparse – "cold" – at baseline. How tumors can be converted from "cold" to "hot" is therefore an important immunological question with significant clinical implications. In mice, CD40 agonists have been shown to generate adaptive immunity, as well as subsequent T-cell infiltration and ICB-sensitivity, against pancreatic ductal adenocarcinoma (PDAC), which is a canonically T-cell sparse cancer. However, responses are not universal. Therefore, elucidation of the restraints on CD40-agonist-induced adaptive immunity remains an important unmet need. To examine the tumor-intrinsic restraints on the generation of adaptive immunity against PDAC in an unbiased and high-throughput manner, we are undertaking a pooled *in vivo* CRISPR screen. To that end, we have generated a clonal fluorophore- and cas9-expressing PDAC cell line and are building a targeted library of sgRNAs.

P24. "Tissue-Resident Memory $CD8^+$ T Cells in the Pancreas and Secondary Lymphoid Organs of Diabetic and Auto-Antibody Positive Organ Donors"

Alberto Japp, Marcus Buggert, Wenzhao Meng, Jay Gardner, Heidi Gunzelman, Maria Golson, Chengyang Liu, Klaus H. Kaestner, Eline T. Luning-Prak, Ali Naji and Michael Betts

Type 1 diabetes (T1D) is caused by the autoimmune destruction of insulin-producing beta cells in the pancreatic islets, resulting in insulin dependency for glycaemia control. Auto-reactive cytotoxic $CD8^+$ T cells are drivers of this destruction, but the *in vivo* functional and phenotypic characteristics of T1D-specific $CD8^+$ T cells remain to be clarified. Here, we directly examined pancreas-draining LN and islets obtained by the HPAP for the presence of resident memory T1D-specific $CD8^+$ T cells (T_{RM}). T_{RM} are characterized by the expression of the tissue retention markers CD69 and CD103, are not found in blood, and are associated with localized allergic and autoimmune diseases of the skin, gut and lungs.

Islet-specific $CD8^+$ T cells can be identified by tetramers in the spleen and pancreas-draining LN of T1D and non-diabetic individuals directly *ex vivo*. These cells have an antigen-experienced phenotype and express the T_{RM} retention markers, without evidence of recent activation. Interestingly, they appear to be non-cytolytic, with low perforin and granzyme B expression. TCR analysis of sorted islet-specific $CD8^+$ T cells from a T1D donor revealed oligoclonality, suggestive of priming and clonal expansion *in vivo*. In addition, $CD8^+$ T cells in pancreatic islets expressed CD69 and CD103 and, in T1D, showed increased levels of proliferation and differential expression of eomes and T-bet.

These observations support a role for $CD8^+$ T_{RM} cells in T1D. Further work will identify the functional and transcriptional properties of these cells during health and disease, providing new insights into the pathobiology of T1D.

P25. "A role for endothelial cell intrinsic IKKα in regulation of immune homeostasis"

Nipun Jayachandran, Kelly A. McCorkell, Athena Patel and Michael J. May

The pro-inflammatory transcription factor NF-κB plays key roles in regulating immune and inflammatory diseases, and is activated through distinct mechanisms named the canonical and non-canonical (NC) pathways. Activation of NF-κB in vascular endothelial cells (EC) promotes secretion of inflammatory cytokines and expression of adhesion molecules that contribute to the initiation and maintenance of inflammatory responses. Although the significance of canonical NF-κB signaling in EC has been extensively studied, the role of NC NF-κB in these cells is not well understood.

Previous findings from our lab have shown that the NC NF-κB pathway is activated in ECs upon Lymphotoxin (LT)β Receptor ligation. To determine the physiological role of this pathway, we developed mouse models targeting IKKα in ECs early in embryonic development by crossing IKK$\alpha^{f/f}$ mice with Tie2-cre animals. The resulting mice lack lymph nodes (LNs) and displayed impaired peripheral B cell maturation. As hematopoietic progenitors are derived from Tie2+ hemogenic endothelial cells, we found that both the EC and hematopoietic compartments lack IKKα in these mice. Despite lacking all peripheral lymph nodes, the spleens were overtly intact in IKKα^{Tie2} mice. Our experiments using fluorescence microscopy and flow cytometry have revealed a reduction in the cellularity of the marginal zone and B cell compartments with significant loss of metalophilic and marginal zone macrophage subsets that are bordering these regions. This resembles the phenotype exhibited by hematopoietic specific IKKα deletion in mice. To determine the EC intrinsic role of IKKα, we crossed IKK$\alpha^{f/f}$ mice with VE-CAD cre and LYVE1 Cre targeting the NC NF-κB pathway in blood or lymphatic endothelial cells (BECs or LECs) respectively. In both these mice, the splenic cellularity and architecture were intact. However, IKKα^{LYVE1} mice lacked all peripheral LNs whereas IKK$\alpha^{VE\text{-}CAD}$ mice had normal

peripheral LN numbers but displayed reduced B cell follicles in LNs. We are currently investigating the mechanisms leading to the loss of B cell follicles in these animals. Together, our data indicate that the NC NF-κB pathway in LECs is critical for LN organogenesis. However, IKKα in BECs is required for the development of B cell follicles within LNs, whereas the maintenance of B cell numbers and overall splenic cellularity requires IKKα in the hematopoietic cells. These findings therefore reveal separate functions for IKKα in distinct hemogenic EC-derived compartments regulating immune homeostasis and LN development.

P26. "Investigating the role of trithorax group protein ASH1L in hematopoietic stem cell homeostasis"

Gloria Jih, Ivan Maillard

The essential and evolutionarily conserved trithorax group (trxG) gene Absent, small or homeotic 1-like (Ash1l) contributes to mammalian development and was discovered by our group to be critical for the quiescence and self-renewal of hematopoietic stem cells (HSCs). Because the Drosophila homolog Ash1 positively regulates Hox genes by H3K36 methylation to ensure proper body patterning, the function of mammalian ASH1L is widely attributed to its SET domain-mediated H3K36 methylation. Surprisingly, we found the SET domain to be dispensable for HSC homeostasis, and ΔSET mice are fertile and develop rather normally, suggesting that critical functions of ASH1L are independent of its enzymatic activity. We hypothesize that ASH1L serves to recruit transcriptional regulators and/or chromatin remodelers to genes regulating HSC homeostasis, and are performing proteomic and genomic analysis to identify ASH1L's protein interactors and native gene targets. Our results will reveal how evolutionarily conserved epigenetic regulators adapt its function for mammalian development.

P27. "The early signaling events of a TLR-dependent checkpoint governing B cell responses to DNA-containing antigens"

Vishal J. Sindhava, Arpita Myles, Michael A. Oropallo, Krishna Moody, Kojo Elenitoba-Johnson1, Michael P. Cancro

Central tolerance mechanisms eliminate autoreactive B cells during development, but some autoreactive B cells can escape these mechanisms. Additional intrinsic tolerance mechanisms must exist to impede the outgrowth of activated, autoreactive B cells. TLR signaling can contribute to the activation and proliferation of B cells and are linked to autoimmune disease. Unmethylated CpG DNA, a ligand for TLR9, is found in self DNA and DNA-binding antibodies are a shared feature of a number of autoimmune diseases. Self-antigen complexed with CpG-containing self-DNA activates B cells for short-term proliferation. However, activated cells soon enter cell cycle arrest followed by mitochondrial cell death. To diagnose the signaling pathways governing this peripheral tolerance mechanism we used a phosphoproteomics approach early after activation of B cells with antigen+CpG DNA complexes. In our screen, we identified differential phosphorylation of Bcl6 and p300. In addition, we calculated a decrease in kinase activity of IRAK4, downstream of TLR9, when B cells are stimulated with antigen+CpG DNA complexes. We are following up on these directions to determine why B cells stimulated with antigen complexed with self-DNA arrest in cell cycle and undergo apoptosis.

P28. "GD2-directed immunotherapy extends survival in a pre-clinical model of high-risk neuroblastoma"

Spyridon Karageorgos, Gabrielle M. Ferry*, Priya Khurana, Annette Vu, Michael D. Hogarty, and Hamid Bassiri

Despite recent FDA approval of dinutuximab (chimeric mAb against GD2, a disialoganglioside expressed on neuroblasts), 5yr overall survival in children with high- risk neuroblastoma (HRNB) is <60%, emphasizing the need for improved therapies. To better delineate the mechanisms of anti-GD2 therapy and to identify potential synergistic therapies, we used *TH-MYCN*$^{+/+}$ mice, an immune-competent pre-clinical model of HRNB. It is postulated that dinutuximab engages NK cell Fc receptors and induces ADCC of GD+ neuroblasts. Indeed, we find a large frequency of NK cells in *TH-MYCN*$^{+/+}$ tumors. Moreover, use of anti-GD2 results in increased blood frequencies of Ly49H-expressing NK cells, when compared to isotype-matched mAb treatment. Importantly, we observe a significant survival advantage associated with anti-GD2 therapy, with 50% of mice showing long-term survival even after treatment cessation. Currently, we are analyzing the effects of therapy on the frequencies, phenotypes, and functions of intratumoral NK cells, as well as associated changes in the tumor environment. We hope these studies will identify additional targets to improve the outcomes of HRNB.

P29. "The HMG transcription factor TOX induces a transcriptional and epigenetic program of CD8+ T cell exhaustion in chronic infection and cancer"

Omar Khan, E. John Wherry

Exhausted CD8$^+$ T cells in chronic infections and cancer are characterized by loss of optimal function, high co-expression of inhibitory receptors and extensive transcriptional changes compared to functional effector or memory CD8$^+$ T cells. Because of their role in cancer and infections, T_{EX} are now important clinical targets of checkpoint blockade and other immunotherapies. Recent epigenetic studies have demonstrated that T_{EX} are a distinct immune lineage. However, the mechanisms that govern the transcriptional and epigenetic events of T_{EX} fate commitment remain unknown. Here, we identify the HMG-box protein TOX as a master regulator of the T_{EX} lineage. TOX is largely dispensable for T_{EFF} and T_{MEM} formation, but in the absence of TOX, T_{EX} do not form. TOX is induced by calcineurin and NFAT2 and then operates in a feed-forward loop to become calcineurin independent and durably expressed at high levels in T_{EX}. TOX interacts with the histone modifying complex HBO1 and its key enzymatic subunit, the acetyl-transferase Kat7, providing a mechanism for T_{EX} lineage-specific epigenetic changes. Thus, high and sustained expression of TOX causes lineage commitment to T_{EX} by translating persisting TCR stimulation cues into a distinct T_{EX} transcriptional and epigenetic developmental program.

P30. "Exploring the immunometabolism and anti-tumor properties of invariant natural killer T (iNKT) cells in neuroblastoma"

Priya Khurana, Hamid Bassiri

Neuroblastoma (NB) is a pediatric tumor of the sympathetic nervous system that contributes to significant childhood cancer mortality. Our lab immunophenotyped tumors

from an autochthonous murine model of NB (TH-MYCN$^{+/+}$) to identify candidate cells for immunotherapy. We found a significant frequency of invariant natural killer T (iNKT) cells relative to conventional T cells (T_{CONV}). iNKT cells have innate-like anti-tumor properties and engage direct tumor cytotoxicity, and predict improved survival in human NB tumors. We postulate that the higher frequency of iNKT cells in NB may imply a unique adaptability to the tumor microenvironment (TME). In T_{CONV}, effector function in the TME is linked to underlying metabolism. However, the metabolic properties of iNKT cells are unknown yet could provide critical insight into their anti-tumor activity in the TME. Here, we utilize a conjugate molecule to activate intratumoral iNKT cells in NB and define the transcriptional metabolic and functional profile of baseline and stimulated iNKT cells. Collectively, we aim to determine novel cellular properties of iNKT cells to allow for more effective NB immunotherapy.

P31. "Neo-epitopes generated by alternative splicing can be targeted by TCR-based adoptive T cell therapy"

Gloria Kim, James Riley

A key challenge to TCR-based forms of adoptive T cell cancer therapy is finding a TCR that robustly directs T cells to tumors but not healthy tissue. To identify promising tumor targets, we employed quantitative mass spectrometry of HLA-A2 bound peptides presented by tumors and related healthy tissue. We identified an epitope within COL6A3 gene (FLLDGSANV) that is presented by tumors but not by healthy tissue. This epitope falls within exon 6 of COL6A3 which is spliced out by healthy cells but is spliced in within a wide range of solid tumors including colon, ovarian, prostate and pancreatic ductal adenocarcinoma, making this an attractive immunotherapy target. Three TCRs with varying affinity for $COL6A3_{642-650}$ (FLLDGSANV) were isolated and evaluated for their ability to redirect T cells to tumors. Additionally, we identified related HLA-A2 binding peptides within the human exome. Of the 8 related sequences, only 1 from COL6A1 provoked a TCR response from the highest affinity COL6A3 TCR that was not observed in the other lower affinity TCRs. In vivo, T cells expressing the medium affinity COL6A3 TCR were able to control tumors expressing the cognate antigen but had no activity against tumor expressing the COL6A1 target. These data indicate that differential alternative splicing products between tumor and healthy tissue will generate promising immunotherapy targets.

P32. "Longitudinal dynamics of follicular CD4 T cells in acute SIV infection"

Leticia Kuri-Cervantes, Claire Deleage, Emily R. Roberts, Heidi M. Gunzelman, Diane G. Carnathan, Guido Silvestri, Michael R. Betts

Follicular T helper CD4+ (Tfh) cells play a critical role in germinal center (GC) formation and B cell maturation. GCs in lymph nodes (LN), particularly within Tfh cells, are sites for preferential SIV infection and replication. Changes in Tfh cells in early acute SIV infection may be a major determinant in the development effective antibody-mediated control of SIV infection.

Eighteen rhesus macaques were intravenously infected with SIVmac251 and underwent staggered necropsy during acute infection. Tfh cells from surface LN (sLN), mesenteric LN (mLN) and spleen were immunophenotyped. We examined mLN to quantify and localize

viral RNA (vRNA), and performed gene expression and pathway enrichment analyses on sorted Tfh cells from LNs in resting and stimulated conditions.

The frequency of Tfh cells was affected by day 10 post-infection (p.i.) and partially rebounded in all tissues. Plasma viremia (pVL) peaked day 10 p.i., but vRNA in mLNs was detectable by day 5 p.i. Tissue vRNA was increased until 90 days p.i. and was not preferentially found within the follicles. Transcriptional profiling of Tfh-related genes showed profound modulation of cytokine production and inflammatory pathways. We observed decreased Tfh responsiveness to stimulation by day 5 p.i., which was partially recovered after 20 days p.i. irrespective of the increasing vRNA found mLNs.

SIV infection has a profound effect in Tfh across tissues since acute infection. This suggests a temporal decrease in Tfh ability to provide B cell help during early stages of infection associated with high levels of viremia, that may directly impact or delay the early induction of SIV-specific antibody production.

P33. "Single-Cell RNA-Sequencing of Peripheral Blood Mononuclear Cells Reveals Changes in Immune Cell Composition and Distinct Inflammatory Gene Expression Profiles during Idiopathic Multicentric Castleman Disease Flare"

Ruth-Anne Langan, David Fajgenbaum, Taku Kambayashi

Idiopathic multicentric Castleman disease (iMCD) is a rare and deadly hematologic illness involving episodic disease flares with polyclonal lymphoproliferation, systemic inflammation, and multiple organ system dysfunction. With the 10-year mortality rate for iMCD reported as 60%, there is a clear need for additional treatment options. However, the development of next generation therapeutics is challenging as the etiology and pathological cell types involved in iMCD are largely unknown. To identify cellular drivers of iMCD, we applied single-cell RNA-sequencing (scRNA-seq) technology to investigate bulk PBMCs isolated from an iMCD patient during a short remission period following the first disease flare (partial remission) and at the start of his second flare (flare 2). We utilized the Cellranger pipeline (10x Genomics, v.2.1.0) for aggregation of single-cell transcriptomes and Loupe Cell Browser (10x Genomics, v.2.1.0) for initial analysis of 20,135 recovered cells from partial remission (16,283 mean reads/cell, 799 median genes/cell) and 19,322 recovered cells in flare 2 (17,327 mean reads/cell, 823 median genes/cell). Initial analyses revealed changes in the composition and frequency of immune cell subsets between the two samples. For example, we found that plasma cells increased 7-fold in number during flare 2. Interrogation of gene expression profiles of immune cell clusters identified highly activated $CD8^+$ T cells which increased in frequency during flare 2 and are characterized by an inflammatory gene signature including elevated expression of perforin and granzyme. Additionally, we identified inflammatory gene signatures within the myeloid cell compartment during flare, including elevated expression of S100 family members. To our knowledge this is the first application of cutting edge single-cell sequencing technology to PBMCs obtained from an iMCD patient in flare and remission. This dataset demonstrates changes in gene expression within multiple immune cell populations during iMCD flare and provides a novel resource for understanding gene expression and cell population changes in Castleman disease.

P34. "Human Genetics Reveals GIMAP5 as a novel member of the Ragulator Complex"

Michael Leney-Greene, Helen Su, Michael Lenardo

In this study, we harness Whole Exome Sequencing (WES) to interrogate the underlying genetic cause in a patient cohort suffering from a Mendelian disease of immune dysregulation characterized by severe lymphopenia, splenomegaly, anemia, thrombocytopenia and liver failure. Bioinformatic analysis revealed novel mutations in a small GTPase that led to a near complete loss of mature protein in patient cells. Animal models lacking these genes develop a disease remarkably similar to that observed in the human patients, however, the molecular role of this GTPase in the immune system remains a fundamental gap in our knowledge. We harness a biotin-ligase system to define the GIMAP5 interactome and show that GIMAP5 is a novel, T cell specific member of the regulator complex. While we did not observe any *in vitro* alterations of mTORC1 activity, treatment with inhibitors of mTORC1 led to remarkable clinical improvements in our mouse model and also one human patient establishing it as a possible treatment. Our future work will focus on dissecting the role of this interaction in T cell biology.

P35. "Regulatory T cell dysfunction in a mouse model of pregnancy loss"

Emma L. Lewis, Guillermo O. Barila, Paige M. Porrett, Michal A. Elovitz

One of the proposed mechanisms of unexplained early pregnancy loss is maternal immunologic rejection of the fetus. However, the specific immune mechanisms causing rejection have not been described. Regulatory T cells (Tregs) are necessary to maintain maternal-fetal tolerance, and prior studies have demonstrated that systemic Treg depletion leads to fetal demise. We hypothesize that mechanisms of fetal rejection involve qualitative Treg dysfunction, in addition to quantitative defects.

We utilized a mouse model of fetal loss, CBA female mice mated with DBA/2 males, to investigate a more mechanistic understanding of Treg function in the setting of fetal rejection. Pregnant CBA and C57BL/6 mice were sacrificed at embryonic day 14 (E14) and assessed for fetal resorption. Maternal spleen, blood, decidua and placenta were collected for analysis by flow cytometry and ELISA. In subsequent experiments, T cells from the spleens of virgin and pregnant CBA and C57BL/6 mice were sorted and used for *in vitro* assays.

The resorption rate of CBA pregnancies is 18-fold higher than that of C57BL/6 pregnancies (CBA: 21.1%, C57BL/6: 1.14%, p=0.0005). Virgin CBA and C57BL/6 female mice have equivalent splenic Treg population sizes. During pregnancy, C57BL/6 splenic Tregs increase from 8.0% to 20.5% of CD4+ T cells (p=0.0003), while CBA mice show no Treg expansion during pregnancy. Similarly, virgin CBA and C57BL/6 female mice have equivalent levels of splenic CD8+ T cells, but C57BL/6 mice have decreased levels of splenic CD8+ T cells during pregnancy (p=0.0003), while CBA CD8+ T cell levels do not change. Specific to the maternal-fetal interface, placentae from CBA pregnancies have fewer Tregs (p=0.023) and more IFNγ protein expression (p=0.0005) than placentae from C57BL/6 pregnancies. Tregs and CD8+ T cells from CBA mice also demonstrate altered functional ability *in vitro*: 1) Tregs from virgin C57BL/6 and CBA mice have equivalent suppressive activity, but Tregs from pregnant CBA mice have less suppressive activity than those from pregnant C57BL/6 mice (p=0.004). 2) Unstimulated CD8+ T cells from CBA mice proliferate less than C57BL/6 CD8+ T cells, both in pregnant (p=0.007) and virgin animals (p=0.0003).

Collectively, these results indicate that CBA mice have an altered immune state that is further changed by pregnancy. The functional immune changes found in CBA mice likely contribute to the high rate of fetal loss. Correlating these immunological changes in CBA mice to humans with recurrent pregnancy loss may lead to new understanding of this adverse reproductive outcome.

P36. "Immune infiltration is heterogeneous in pancreatic ductal adenocarcinoma"

Joey H. Li, Jae W. Lee, Yan Li, Ishir Seth, Devora Delman, Gregory L. Beatty

Cancer immunotherapy has shown remarkable efficacy for some patients with melanoma and non-small cell lung cancer. However, there has been limited success with immunotherapeutic approaches in GI malignancies, such as pancreatic ductal adenocarcinoma (PDAC). The tumor microenvironment has been suggested to be a major barrier to immunotherapy in PDAC due to its predominance of immunosuppressive myeloid cells and T regulatory cells (Tregs) and a lack of effector T cells. Past efforts to interrogate this immune microenvironment have relied on transcriptional profiling with multiplex immunofluorescence microscopy to identify immune signatures within tumors. However, the spatial heterogeneity of immune cell infiltrates is vast and represents a major challenge to interpreting transcriptional and immune signatures. Here, we have used multiplex chromogenic immunohistochemistry (IHC) and RNA *in situ* hybridization (ISH) to identify distinct spatial networks of immune cells and to understand immune infiltration in PDAC. We developed an imaging-computational analysis workflow and found that T cell and myeloid cell infiltration is heterogeneous both within tumors and across patient lesions. Subsequently, we performed laser capture microdissection (LCM) and RNA-sequencing analysis of tumor and stromal tissue areas. This analysis shows that tumor and stromal cells harbor distinct transcriptional signatures with most immune-related genes, including cytokines, chemokines, and interleukins, expressed by stromal cells rather tumor cells. Moreover, we have found that immune infiltrates form cellular communities in PDAC tumors that can be categorized based on the magnitude of $CD14^+/CD68^+$ myeloid and $CD8^+$ T cell infiltrates. We also find that specific immune regulatory molecules with the stroma correlate with $CD8^+$ T cell infiltration. These results suggest that multiple mechanisms may underlie the complex spatial immune networks seen in PDAC and provide insight into efforts to unravel immune suppression for realizing the potential of immunotherapy in this disease.

P37. "Systemic dysregulation of type 1 classical dendritic cells is established early in pancreatic oncogenesis and reversible through CD40 agonism"

Jeffrey H. Lin, Robert H. Vonderheide

Pancreatic ductal adenocarcinoma (PDAC) ranks as the third-leading cause of cancer-related deaths in the United States with a five-year survival rate of 9%. While immune checkpoint blockade (ICB) has revolutionized treatment of many metastatic diseases, cancers like PDAC that lack tumor-infiltrating CD8+ T-cells remain refractory to ICB. To determine whether this phenotype is due to defective CD8+ T-cell priming by antigen cross-presenting type 1 classical dendritic cells (cDC1s), we use the KPC mouse model of PDAC driven by *Pdx1-Cre* $Kras^{LSL\text{-}G12D/+}$ $Trp53^{LSL\text{-}R172H/+}$ to interrogate how type 1 classical dendritic cells (cDC1s) change over the course of pancreatic oncogenesis. In the present study, flow cytometric analysis reveals a progressive and global decline in cDC1 numbers

and maturation that begins in mice bearing preinvasive pancreatic intraepithelial neoplasias (PanINs). This dysfunction worsens upon progression to malignancy. However, administration of CD40 agonist to tumor-bearing mice effectively rescues cDC1 maturation and restores CD8+ T cell priming. Our findings reveal that cDC1s are dysregulated early and prominently in the neoplastic process and position CD40 agonism as an ideal strategy to potentiate response to ICB in resistant cancers like PDAC.

P38. "Coxiella burnetii uses a type IV secretion system to evade human inflammasome activation"

Natasha Lopes Fischer, Sunny Shin

Pathogens are at a constant arms race to evolve strategies for counteracting host immunity. At the cellular level, the host possesses mechanisms to detect pathogenic activity and mount an immune response. Host cytosolic proteins such as the NOD-like receptors and caspases detect and respond to bacterial activity. These proteins form an inflammasome complex, which mediates the secretion of inflammatory cytokines and alerts the body to the infection. Some intracellular bacteria have evolved strategies to escape this host detection. The bacterium *Coxiella burnetii* escapes immune recognition and causes a flu-like disease. It infects macrophages in the lung, where it uses a secretion system to inject bacterial effector proteins into the host cytoplasm. These effectors modulate host cellular processes and allow bacterial replication. My preliminary data suggest that *Coxiella* evades inflammasomes by using its secretion system. Based on this data, I hypothesize that *Coxiella* secretes effectors that suppress inflammasome activation. I will investigate what inflammasomes are evaded by *Coxiella* and identify effectors that suppress this immune response.

P39. "Gnotobiotic mouse model of the early-life microbiome to identify microbes that prevent autoimmune diabetes"

Jean-Bernard Lubin, Michael Silverman

Certain major histocompatibility complex-class II (MHC-II) or human leukocyte antigen-D (HLA-D) alleles dominantly protect from particular autoimmune diseases. For example, expression of the MHC-II Eα:Eβ (Eα16) complex potently protects NOD mice, which normally lack it, from spontaneous development of type-1 diabetes (T1D). However, the underlying mechanisms remain debated. Genomic analysis revealed NOD and Eα16/NOD mice to host distinct intestinal microbiotas during a critical early window of ontogeny, and transfer of cecal contents from the latter to the former suppressed insulitis. As this protection occurs early in life, we have sought to develop a *simplified community of bacteria derived from pre-weaned, Eα16/NOD mice that will recapitulate the diabetes-protective immunologic impacts of the whole microbiome and model the composition and phenotype of pediatric intestinal microbiome.*

Small Intestine, cecum and large intestine samples from 14 day old, Eα16/NOD mice were cultured and microbes were isolated and analyzed 16s rRNA metagenomic sequencing (MGS). The OTUs cultured represented >99% of the reads sequenced from the organ sites. Candidate microbes were selected from the assembled isolate collection to form a nine-member community designated as Pediatric Community (PedsCom). PedsCom was orally gavaged in to 10 week-old, germ-free (GF) C57BL/6J mice and colonization was

tracked through 16s rRNA MGS of stool samples. Three weeks post gavage, 7 of 9 isolates were still present in the stool. This would indicate we have a stable community for long-term colonization.

To test the phenotypic characteristics of the PedsCom gnotobiotic mouse model, the offspring (F1 generation) were assessed by cecal weights, serum Immunoglobulin (sIg) concentrations and intestinal lumen lymphocyte populations. PedsCom mice cecal weights were significantly lower than GF controls but significantly higher than Specific pathogen free controls, suggesting an intermediate phenotype in our gnotobiotic mouse. Lower serum levels of IgA and IgG1 are hallmarks of GF mice. Microbial colonization in the PedsCom mouse raised sIgA and sIgG levels compared to GF controls. Recapitulation of normal immune components was further assessed by examining the lymphocyte populations of the PedsCom mouse. In particular, we are interested in the proportion of T-regulatory cells (T_{regs}), and microbially-induced *(i-*T_{regs}) generated by PedsCom due to their role in suppressing autoreactive T-cells in autoimmune disease models. When compared to GF controls, PedsCom mice have lower relative proportions of Tregs in the cecum and significantly higher proportions of *i-*T_{regs} in the cecum and large intestine, falling in between GF and SPF controls.

In summary, we have successfully cultured and constructed a pediatric synthetic community that preliminary data suggests intermediate functionality compared to a complete microbiome. Intermediate phenotypes in cecal weights and lymphocyte populations may indicate that our gnotobiotic model functions similarly to an underdeveloped infant microbiome. To our knowledge this is the first gnotobiotic model designed specifically for the purpose of modelling the pediatric intestinal microbiome.

P40. "Translocation of a gut pathobiont drives autoimmunity in mice and humans"

Silvio Manfredo Vieira, Aimee Payne

Host-microbiota interactions in the pathogenesis of autoimmunity remain poorly understood. Here, we show that a gut commensal, *Enterococcus gallinarum*, reaches lymphocytes beyond the gut barrier in the mesenteric lymph node, liver and spleen of lupus-prone (NZWxBXSB)F1 mice. Oral vancomycin treatment suppressed growth of *E. gallinarum* in tissues of these mice, prevented organ manifestations and mortality by lowering pathogenic autoantibodies, Th17 and Tfh cells. Hepatocyte-commensal cocultures revealed induction of the lupus signature cytokine IFN-α as well as the AhR pathway (AhR, Cyp1a1, Cyp1a2) that could be linked to Th17 induction in vivo. *E. gallinarum* monocolonization in germ-free C57BL/6 animals allowed for translocation to internal organs and induction of autoantibodies as well as increase of lamina propria plasmacytoid dendritic cells (pDCs) and systemic Th17 cells. Additionally, RNA-Seq of short-term ileum *E. gallinarum*-monocolonized mice demonstrated induction of molecules related to barrier function (occludin, claudins, Plvap, Axin2), the mucus layer (mucin, Fut2), antimicrobial defence (Reg3b, Defa2), and inflammation and pDC induction (AhR/Cyp1a1, Enpp3). Species-specific PCR of sterilely obtained liver tissue from autoimmune hepatitis and lupus patients revealed also *E. gallinarum*, suggesting similar processes in humans. Accordingly, human hepatocyte-commensal coculture demonstrated production of the same autoimmune- promoting factors as with murine hepatocytes. Collectively, these data indicate that a human pathobiont translocates spontaneously to promote autoimmunity in genetically predisposed hosts, broadening our understanding of autoimmune host-microbiota interactions.

P41. "CD4+ T cells support clearance of Clostridium difficile upon fecal microbiota transplantation"

Rina Matsuda, Isma Zarin, Rebecca Carter, Eric Littmann, Michael Abt

Clostridium difficile is a prevalent nosocomial pathogen causing gastrointestinal disease, potentially leading to life-threatening colitis. Antibiotic treatment renders people susceptible to *C. difficile* infection by disrupting the indigenous gut microbiota. Recently, fecal microbiota transplantation (FMT) has emerged as an effective clinical treatment option. However, the mechanisms that determine successful FMT remain poorly understood. Here, we use a murine model of persistent *C. difficile* infection to demonstrate a critical role for the host immune system in determining FMT efficacy. We have found that in *Rag1*$^{-/-}$ mice lacking T and B cells, FMT cannot resolve *C. difficile* infection, suggesting that adaptive immunity is needed for FMT efficacy. Similarly, mice specifically lacking CD4^{+} T cells cannot resolve infection upon FMT. In these mice, transplanted bacteria do not fully engraft into the colonic environment. Further, mice deficient in T regulatory cells exhibit delayed clearance of *C. difficile* upon FMT. This work is the first to demonstrate a role for host immunity in FMT efficacy, shedding light on fundamental interactions between mucosal immunity and the gut microbiota. Findings may inform treatment of *C. difficile* in immunocompromised patients and have implications in novel applications of microbiota-based therapeutics.

P42. "The E3 Ubiquitin Ligase Itch Restricts Magnitude of Germinal Center B Cell Responses"

Emily Moser, Paula Oliver

The ubiquitin ligase Itch regulates antibody levels and prevents autoimmune disease in mice and humans, yet how Itch regulates B cell fate or function *in vivo* is unknown. We now show that Itch directly limits B cell activity in vivo and in vitro. Itch deficient mice displayed increased antigen-experienced B cells, but pre-immune B cell populations were comparable to controls. In mixed bone marrow (BM) chimeras, Itch functioned within B cells to limit GC B cell numbers and antigen-specific GC B cells and plasma cells after immunization. Proteomic profiling identified and subsequent studies confirmed that Itch regulated mTORC1 activation, proliferation, and glycolysis within B cells cultured in vitro. Finally, in an immunization model, we demonstrated that Itch deficiency in B cells was sufficient to cause increased antigen-specific GC B cells, BM plasma cells, and high affinity serum IgG. These results identify a novel role for Itch in B cells, and they support a model in which Itch limits B cell metabolism and proliferation to suppress GC B cell responses, aligning with Itch's role in preventing autoimmune disease.

P43. "Group 1 Innate lymphoid cell lineage identity is determined by a cis-regulatory element marked by a long non-coding RNA"

Walter Mowel, Jorge Henao-Mejia

Commitment to the innate lymphoid cells (ILC) lineage is determined by *Id2*, a transcriptional regulator that antagonizes T and B cell-specific gene expression programs. Yet how *Id2* expression is regulated in each ILC subset remains poorly understood. We identified a *cis*-regulatory element demarcated by a long non-coding RNA (lncRNA) that

controls the function and lineage identity of group 1 ILCs, while being dispensable for early ILC development and homeostasis of ILC2s and ILC3s. The locus encoding this lncRNA, which we termed *Rroid*, directly interacted with the promoter of its neighboring gene, *Id2*, in group 1 ILCs. Moreover, the *Rroid* locus, but not the lncRNA itself, controlled the identity and function of ILC1s by promoting chromatin accessibility and deposition of STAT5 at the promoter of *Id2* in response to interleukin (IL)-15. Thus, non-coding elements responsive to extracellular cues unique to each ILC subset represent a key regulatory layer for controlling the identity and function of ILCs.

P44. "Role of the human NAIP inflammasome in intestinal cell-intrinsic responses to Salmonella"

Nawar Naseer, Sunny Shin

Non-typhoidal salmonellosis is a leading cause of diarrheal disease. There is a need to better understand the innate immune response to *Salmonella*. *Salmonella* uses type III secretion systems (T3SS) to inject effectors into the host cell cytosol. These T3SSs allow *Salmonella* to invade and replicate within host cells such as intestinal epithelial cells (IECs). Host cells can recognize T3SS components through cytosolic receptors termed NAIPs. Upon sensing their ligands, NAIPs form an inflammasome complex, leading to IL-1 family cytokine secretion and cell death. The NAIP inflammasome is critical for control of *Salmonella* in mice and results in extrusion of infected cells from the intestinal epithelial layer. While mice have several NAIPs, each specific to a particular ligand, humans only have one, hNAIP. In macrophages, hNAIP recognizes bacterial flagellin, the T3SS inner rod, and needle proteins. hNAIP is highly expressed in IECs, but the role of hNAIP in IEC responses to *Salmonella* is unknown. I am testing the hypothesis that IECs use hNAIP to sense S. Typhimurium T3SSs and mediate responses for controlling bacterial infection.

P45. "$CD8^+$ T cells have an intrinsic tendency of becoming exhausted upon K^+ ion efflux"

Niavi C, Cai Z, Attanasio J, Lau CW, Nzingha K, Ekshyyan V, Ngiow S, Stelekati E, Wherry EJ

Ion flow has crucial effects on T cell differentiation. Ion channel dysregulation or loss may result in cancer progression. Also, there has been evidence that tumor necrosis leads to potassium ions efflux to the extracellular fluid inhibiting the function of T cell effectors. Previously, in an effort to detect new druggable immunotherapy targets that are related to exhausted T cells (Tex), we identified KCNA3 potassium channel as a potential candidate. The screening was based on changes in expression following PD-L1 and/or LAG-3 blockade in the LCMV clone 13 chronic infection system. Here, we overexpressed KCNA3 using a retroviral system in LCMV-specific CD8 T cells and tested their response to LCMV clone 13. We found an increase in expression of inhibitory receptors, such as PD1 and 2B4, suggesting that KCNA3 overexpression promotes exhaustion. Further, we found that memory-associated molecules such as Ly108 was expressed in lower levels after KCNA3 overexpression. These data suggest that $CD8^+$ T cells have an intrinsic tendency of becoming exhausted upon K^+ ion efflux. Subsequently, it would be of high interest to test potential inhibitors of this ion channel in order to reverse exhaustion as a cancer immunotherapy approach.

P46. "PmpG and MOMP define circulating human CD4 T cell responses to *Chlamydia trachomatis*"

Laura A. Vella, Kaitlin C. O'Boyle, Alex Musselman, Hualin Li, Sheri Dubey, Kalpit A. Vora, E. John Wherry

C. trachomatis is the leading cause of bacterial STIs worldwide. Its slow clearance and high rate of reinfection indicate that the initial immune response is ineffective. However, risk of reinfection has been shown to decrease with years of exposure, suggesting that development of an effective immune response is possible. Studies in mice suggest that Th1 cells promote clearance of infection, and a focus of human studies has been to identify potential vaccine candidates to elicit these responses. To determine target antigens, we enrolled 100 adolescents from a family planning clinic who were undergoing testing for Chlamydia infection. Of these patients, 13 had active Chlamydia infection, 21 were negative but had prior positive test results, and the remaining had no documented positive tests. We performed *in vitro* PBMC stimulations with recombinant 15-mer overlapping peptide libraries from *C. trachomatis* Hsp60, PmpG, and MOMP. Cytokine responses were assessed by flow cytometry. We identified poly-functional CD4 T cell responses to PmpG and MOMP in a subset of Chlamydia-exposed individuals at frequencies likely missed by less sensitive immunologic assays.

P47. "Common fragile site mapping by mitotic DNA synthesis sequencing

Jacob Paiano, Andre Nussenzweig

Common fragile sites (CFSs) were originally defined as chromosomal regions of frequent DNA breaks on metaphase chromatids following replication stress and are associated with some cancer transformations, copy number variations, and translocations. A pathway of mitotic DNA synthesis (MiDAS) has recently been discovered to mitigate chromosomal instability at some CFSs when DNA is under-replicated upon mitotic entry. MiDAS is typically measured by fluorescent foci of EdU-labeled, newly synthesized DNA, yet the precise genomic locations of these microscopy foci are unknown. This study aims to identify all sites of MiDAS by EdU-labeled DNA sequencing (EdU-seq) so that a comprehensive chromatin characterization of CFSs can be done. We employ EdU-seq after aphidicolin-induced under-replication in mitotic U2OS cells and observe MiDAS at ~24 sites genome-wide, some of which are known CFSs and all of which are very large (~1Mb), lowly transcribed genes. Intriguingly, our data pair well with recently published FANCD2 ChIP-seq, indicating that these EdU-seq MiDAS events are sites of under-replication upon mitosis. Using EdU-seq to more precisely identify MiDAS, we will address why these sites are prone to under-replication, how they are resolved, and the true extent that they reflect CFSs and disease.

P48. "The Neurokinin-1 Receptor pathway modulates the CD16- and CD16+ monocyte subsets"

Pappa Vasiliki, Spitsin Sergei, Douglas D. Steven

Background: Monocytes are a heterogeneous cell population, consisting of CD16- and CD16+ cells. In HIV infected individuals, the CD16+ subset increases individuals to 20-60% from 10-15% in non-infected individuals. CD16+ monocytes are more susceptible to infection with HIV and they transmigrate to the CNS, where they can contribute to the

development of neuroinflammation. Our previous work demonstrates that in HIV infection elevated plasma substance P (SP) levels occur and that SP/Neurokinin-1 Receptor (NK1R) signaling alters monocyte differentiation. To assess the effect of NK1R signaling on monocyte differentiation we performed an RNA sequencing study.

Methods: Primary monocytes (Penn/CHOP CFAR) were treated with SP (RNA sequencing) and/or aprepitant (qPCR). Monocytes were sorted into CD16- and CD16+ populations (CHOP Flow Core). RNA was isolated and submitted for sequencing (CHOP HTS Core). R packages were used to determine differentially expressed genes (DESeq2), analyze gene-set enrichment (GAGE) and identify differential exon usage (DEXseq).

Results: SP treatment upregulated the expression of pro-inflammatory genes, including IL1α, IL1β, IL6, CCL3 and CCL4 and anti-inflammatory genes, including SOCS3 and TNFAIP3. The NK1R antagonist, aprepitant, reduced the expression of IL1α and SOCS3. The peroxisome pathway was upregulated in SP-treated CD16+ compared to SP-treated CD16- monocytes. During SP treatment, alternative splicing occurs in a distinct set of genes, implicated in inflammasome activity in CD16+ cells.

Conclusions: We confirmed the effect of SP on inflammation and identified upregulated genes and pathways, previously unrecognized in the context of SP treatment of monocytes. We also observed a distinct effect of NK1R signaling on the regulation of genes involved in infammasome activity in CD16+ monocytes. We will study these pathways to understand their contribution in immunomodulation and HIV neuroinflammation. Finally, we will assess the effect of aprepitant on the identified pathways and evaluate its potential as a therapeutics of neuroHIV.

P49. "Notch signals delivered *in trans* to antigen by specialized secondary lymphoid organ fibroblastic stromal cells critically regulate T cell immune responses"

Eric Perkey, Léolène Carrington, Ivan Maillard

Notch signals from secondary lymphoid organ (SLO) fibroblastic stromal cells drive lethal T cell alloreactivity and graft-versus-host-disease (GVHD) after high-intensity myeloablatice conditioning. However, little is known about regulation of Notch ligands in fibroblasts and if they are important sources in other immune contexts. Expression of the Notch ligand Delta-like4 (Dll4) was observed in $CD157^+$ fibroblastic reticular cells, $MAdCAM1^+$ marginal reticular cells, and $CD21^+$ follicular dendritic cells traced by the *Ccl19-Cre* transgene. Allotransplanation drove Dll4 upregulation in the fibroblastic but not other cellular compartments and was potentiated by the intensity of myeloablative conditioning. Still, in GVHD models not requiring myeloablative conditioning (parent to F1 transplantation), fibroblastic cells remained the dominant source of Notch signals driving lethal T cell alloreactivity. However, conditional deletion of MHC Class II in fibroblasts did not protect mice from $CD4^+$ T cell mediated GVHD suggesting that Notch can be delivered by a separate cellular source than antigen. To further explore the cellular source of Notch ligands, we studied dendritic cell immunization loaded with a MHC Class I restricted ovalbumin-peptide antigen. Even when antigen-pulsed dendritic cells expressing high levels of Dll4 were adoptively transferred, Notch ligands expressed in fibroblastic stromal cells were the critical cellular source to drive $CD8^+$ effector T cell differenation. The findings challenge the paradigm that antigen-presenting cells are the exclusive sources of Notch signals in T cell immunity. Instead, we propose a model where specialized subsets

of fibroblastic cells in spleen and lymph nodes provide regulated microenvironmental licensing of T cell response to antigen presenting cells by providing Notch signals *in trans*.

P50. "PD-1 limits $CD8^+$ T cell activation during the acute phase of *Toxoplasma gondii* infection"

Joseph Perry, Christopher Hunter

Blockade of the inhibitory receptor ligand PD-L1 during the chronic phase of infection with *Toxoplasma gondii* increases the functionality of $CD8^+$ T cell responses and result in reduced parasite burden. However, the impact of PD-1 signaling on the immune response to *T. gondii* during the early phase of infection is unknown. The data presented here reveal that acute infection with *T. gondii* results in a STAT1 mediated upregulation of PD-L1 on effector T cells and bystanders. PD-L1 blockade during the acute phase of toxoplasmosis resulted in an increased proportion of activated $CD8^+$ T cells ($CD69^+$, LFA-1^{hi}) accompanied by an increased proportion of polyfunctional $CD8^+$ T cells (IFN-γ, Granzyme B^+) without increasing immune pathology. Unexpectedly, this intervention resulted in a significant increase in the expression of inhibitory receptors such as PD-1, TIM3, and CTLA-4 on the long-lived T-intermediate (Tint, $CXCR3^+$/$KLRG1^+$) $CD8^+$ T cell population. These datasets suggest PD-1 signaling in the early phase of *T. gondii* infection constrains $CD8^+$ T cell effector differentiation and expansion, potentially preserving Tint subsets in the chronic phase of infection.

P51. "Determining the mechanism of TNF-mediated defense against *Legionella pneumophila* infection"

Tzvi Pollock, Sunny Shin

Intracellular bacteria are responsible for significant disease burden every year. Successful control of these organisms by the host relies on the inflammatory cytokine Tumor Necrosis Factor (TNF). While TNF is known to protect against many intracellular infections, and the molecular mechanisms of TNF signaling are understood in sterile contexts, the precise modes through which TNF defends the host against bacterial infection remain unclear. This research aims to elucidate the mechanism of TNF in intracellular infection. In this project the bacterium *Legionella pneumophila*, causative agent of Legionnaire's Disease, acts as a model intracellular pathogen. Infection of bone marrow derived macrophages and live mice are used to explore the effect of TNF on control of infection. Thus far we have shown that TNF is required for restriction of bacterial replication both *in vitro* and *in vivo*. We have also demonstrated that TNF is required for optimal production of the pro-inflammatory cytokines IL-1α, IL-1β, and IL-6. In addition, the protease CASP8 appears to be necessary for control of *Legionella* infection and may be the mode through which TNF restricts replication.

P52. "Developing a DNA vaccine for canine distemper virus"

Sophia Reeder, Emma Reuschel, Kun Yun, Kevin Kim, La'Toya Latney, Tim Georoff, David Weiner

Canine Distemper Virus (CDV) is a RNA virus which is highly contagious by inhalation or by contact with infected bodily fluids. CDV presents a major threat to endangered animal

populations. Development of a robust non-live vaccine is important for the protection many endangered species. The goal of this project is to develop an optimized DNA vaccine to provide protection against CDV through induction of an antibody response as well as cell mediated immunity (CMI). Animals were immunized with DNA encoded viral proteins and studied for immune induction. Serum was collected after each immunization and tissues harvested for immune analysis one week after the final immunization. ELISPOTs were used to assess the CMI elicited by vaccination. Microscopy and ELISAs were used to assess antibodies by the vaccine. We show that a novel synthetic DNA CDV vaccine elicits both humoral and CMI responses against multiple CDV antigens. Overall, our data suggests that a DNA vaccine against Canine Distemper Virus is important for further study to provide an alternative safe and potent vaccine to protect against Canine Distemper in high risk populations in zoo and conservation settings.

P53. "Investigating the Antiviral Role of STING during Enteric RNA Virus Infection in *Drosophila*"

Elisha Segrist, Beth Gold, Sara Cherry

The intestine serves as a physical and immune barrier to protect against infection by enteric pathogens. The Cherry lab uses *Drosophila* as an in vivo model of human and mosquito enteric infection to understand the intestinal innate immune response and how it is influenced by the composition of the microbiota. Sindbis virus is a mosquito-borne virus that is transmitted to vectors orally during a blood meal. My preliminary data suggest the *Drosophila* homologue of Stimulator of Interferon Genes (STING) is antiviral against Sindibs virus within the intestine. Mammalian STING is activated by binding cyclic dinucleotides (CDNs) produced endogenously by cyclic GMP-AMP synthase or exogenously by bacteria. Metagenomic data reveal that Drosophila commensal bacteria encode CDN synthases indicating the microbiota could provide an exogenous CDN pool to prime basal STING activity from subsequent viral infection. Indeed, my preliminary data suggests exogenous feeding of cyclic dinucleotides to flies lacking their microbiota protects against enteric Sindbis virus infection. Moreover, I found that STING is antiviral through the activation of autophagy and inflammatory NF-kB activation. This work sheds light on the ancient cGAS-STING defense pathway and defines how additional microbiota-derived products, such as CDNs, may prime intestinal immunity.

P54. "Investigating nuclear organization of the inactive X during dynamic maintenance of X Chromosome Inactivation"

Isabel Sierra, Montserrat Anguera

Chromatin organization in the nucleus is highly regulated to provide precise control over gene expression. The most dramatic example of the relationship between chromatin structure and gene regulation is X Chromosome Inactivation (XCI). XCI epigenetically converts one active X chromosome into a transcriptionally silent inactive X (Xi) in female mammals, to equalize dosage between the sexes. XCI is initiated by spreading of the long non-coding RNA Xist in *cis* which re-organizes the Xi into a compact, bipartite structure, and targets the Xi to the nuclear periphery. Maintenance of the Xi must be achieved to prevent aberrant expression of X-linked genes, which is observed in autoimmune diseases and cancers. The current paradigm of XCI maintenance is stable association of Xist RNA and heterochromatin marks on the Xi in all female somatic cells. Recently, we found a

novel mechanism of XCI maintenance in female lymphocytes. In naïve female B cells, Xist RNA is not localized to the Xi, but surprisingly, after stimulation Xist RNA and heterochromatin marks robustly return to the Xi. One factor necessary for the localization of Xist RNA to the Xi after B cell stimulation is the transcription factor Yy1, as loss of Yy1 abrogates the localization of Xist RNA to the Xi in stimulated B cells. We identified Yy1 interacting proteins in female naïve and activated B cells with roles in nuclear structure and organization, some of which regulate XCI. We have developed a novel allele-specific imaging system using DNA FISH to quantify the compaction and movement of the Xi during lymphocyte activation. In addition, we are testing the function of YY1-interacting proteins for Xi compaction, movement, and tethering at the nuclear periphery for XCI maintenance. These experiments will elucidate novel pathways for XCI maintenance specific to immune cells.

P55. "Blood meal regulation of midgut immunity and metabolism by the steroid hormone 20-hydroxyecdysone (20E) promotes commensal expansion in *Anopheles gambiae*"

Sarah Sneed, Michael Povelones

The midgut is the first tissue barrier blood meal-acquired pathogens encounter in the mosquito. Therefore, understanding blood meal-specific signals that regulate the midgut epithelium is critical for identifying novel mechanisms important during infection. Midgut commensal bacteria dramatically expand during blood feeding driving a concomitant reduction in bacterial diversity. We find that injection of 20E, a steroid hormone produced shortly after blood feeding and essential for reproduction, induces comparable changes in bacterial quantity and diversity in *An. gambiae*. We characterized the microbial communities present after 20E injection and demonstrate that they are the same as those that expand during blood feeding. RNA-Seq was performed on midguts to identify genes regulated both by 20E-treatment and blood feeding. The majority of target genes identified are involved in metabolism and immune signaling. We hypothesize that a subset of these targets are directly responsible for a tolerating signal that allows the bacteria to expand post-blood meal and may simultaneously increase susceptibility of the midgut epithelium to pathogen infection.

P56. "Trib1 regulates eosinophil lineage commitment and identity by restraining the neutrophil program"

Sarah J. Stein, Ethan A. Mack, Kelly S. Rome, Lanwei Xu, Gerald B. Wertheim, Rossana C. N. Melo, Warren S. Pear

The pseudokinase Trib1 is an important regulator of granulocytes; knockout mice lack eosinophils and have increased neutrophils. Yet, how Trib1 regulates cellular identity and function during granulopoiesis is not understood. We found that Trib1 regulates granulocyte precursor lineage commitment and mature eosinophil identity. Trib1 deletion in HSCs reduced the size of the EoP pool and increased neutrophils; deletion following eosinophil lineage commitment blunted the decrease in EoPs without increasing neutrophils. Trib1-deficient mice also expanded a stable population of $Ly6G^{+}$ eosinophils that retained neutrophilic characteristics and functions. We identified an uncharacterized role for IL-5 in driving both eosinophil and neutrophil differentiation from the GMP; Trib1 suppressed the neutrophil gene program in lineage-committed eosinophil precursors in response to IL-5 signaling. Together, our findings show that Trib1 functions at two distinct

stages to guide eosinophil lineage commitment from the GMP and suppress the neutrophil program, promoting eosinophil terminal identity and lineage fidelity.

P57. "Novel immunosuppressive myeloid cell populations detected in PDAC with high dimensional mass cytometry and multiplex immunohistochemistry"

Meredith Stone, Yan Li, Lindsey Shallberg, Joey Li, Devora Delman, Kathleen Graham, Jessica Wong, Anwar Murtaza, Jinqi Liu, Murali Gururajan, Gregory Beatty

Introduction: Pancreatic ductal adenocarcinoma (PDAC) has demonstrated remarkable resistance to immune-based therapies. This resistance may be due to the microenvironment that surrounds PDAC cells and that is characterized by an abundance of myeloid cells, a scarcity of effector T cells and a dense fibrotic reaction. The tumor microenvironment can be a critical regulator of T cell immunosurveillance in cancer and in PDAC, has been shown to provide malignant cells with mechanisms for immune evasion and in essence, a site of "immune privilege". Overcoming T cell exclusion in PDAC by targeting the tumor microenvironment has therefore emerged as a critical "next step" to realizing the potential of immunotherapy in this disease. Yet, a comprehensive understanding of the various subsets of immune cells and their spatial relationship to malignant cells is lacking. Here, we performed high-dimensional single cell analysis to define the complexity of the myeloid cell reaction to PDAC using a genetic model of this disease.

Methods: We used the $Kras^{G21D/+}$; $Trp53^{R172H/+}$; Pdx-1 Cre (KPC) mouse model of PDAC, which spontaneously develops tumors that recapitulate many of the features of human disease, including a near absence of T cells and a robust infiltration of myeloid cells. Polyclonal pancreatic cancer cell lines were established from spontaneous KPC tumors, and cell lines were orthotopically implanted into syngeneic C57BL/6 mice. The pancreas (containing tumor) and spleen were collected for mass cytometry analysis to examine multiple T cell and myeloid cell markers. Human and murine tissues were formalin-fixed and paraffin-embedded for analysis by immunohistochemistry to define the spatial distribution of myeloid cell populations.

Results: In both human and murine PDAC, we identified multiple myeloid cell populations based on their spatial location within tumors. Specifically, myeloid cells were found adjacent to $CK19^{+}$ tumors, adjacent to $CD31^{+}$ blood vessels, or trapped within the stroma that surrounds malignant cell nests. Using CyTOF, we then identified 25 distinct leukocyte subsets in the spleen of tumor-bearing mice including prominent myeloid cell populations that were expanded in the setting of PDAC development and differed in their expression of CD11b, Ly6G, Ly6C, CD11c, F4/80, Ki67, Arginase1, and PDL1. In addition, we identified 21 unique leukocyte subsets in PDAC tumors including seven myeloid cell subsets that differed primarily in their expression of arginase1, PDL1, CD206, CD169, MHCII, iNOS, GITR, CD115, and Ki67. Each myeloid cell population that was identified in orthotopic tumors was also observed in spontaneous tumors at similar frequencies. Ongoing studies are evaluating the transcriptional signatures of each myeloid cell subset.

Conclusion: Our studies highlight the remarkable heterogeneity and spatial complexity of the leukocyte response to PDAC. In addition, we have identified several novel populations of myeloid cells that emerge in the setting of PDAC development and that may be important targets for improving outcomes to immunotherapy in this disease.

P58. "DDX56 is antiviral against alphaviruses and binds Chikungunya virus RNA during infection"

Frances Taschuk, Sara Cherry

Chikunguya virus (CHIKV) is an emerging arthropod-borne virus in the alphavirus group, and causes outbreaks around the world. We performed an RNAi screen and found that the nucleolar helicase DDX56 has an antiviral role in alphavirus infection. We have found that DDX56 is antiviral against Chikungunya virus, as well as the related alphaviruses Sindbis and O'Nyong'Nyong. Alphaviruses are known to be controlled by type I interferons, but we found that DDX56 is dispensable for interferon signaling. Therefore, we hypothesized that DDX56 exerts its antiviral activity through binding to viral RNA. We used CLIP-Seq of endogenous DDX56 to identify interacting RNAs, and found that a region of the CHIKV genome near the 5' end of nsP4 is bound by DDX56. While nsP4 is produced as part of a polyprotein (nsP1-4), its levels are regulated by degradation of free nsP4 and, in some alphaviruses, by readthrough of an opal stop codon at the end of nsP3. The region we identified is just downstream of nsP3, suggesting that DDX56 attenuates CHIKV infection by interfering with translation of nsP4, and we are currently defining the mechanism by which DDX56 binding to this region of the genome impacts viral protein expression and replication.

P59. "The role of MHC class II on lung type II alveolar cells during influenza virus infections"

Annie Toulmin, Laurence Eisenlohr

Influenza virus (flu) infections cause 3-5 million cases of severe illness and hundreds of thousands of deaths annually. $CD4^+$ T cells are critical regulators of the immune response to flu viruses. By convention, these $CD4^+$ T cell responses are shaped by recognition of peptide/MHCII complexes on the surface of hematopoietically-derived "professional" antigen presenting cells. However, ~1/3 of all MHCII expressing cells in the lung at homeostasis are nonhematopoietic in origin; these include type II alveolar cells (AECIIs), epithelial cells that are the main target of flu infection *in vivo*.

We have begun to explore the possible function of MHCII on AECIIs during flu infections. We find that despite constitutive MHCII expression, flu infected AECIIs are unable to stimulate flu-specific $CD4^+$ T cell hybridomas *in vitro*. Furthermore, AECIIs do not present two model MHCII-restricted epitopes *in vivo*. A potential explanation for poor MHCII presentation is that AECIIs lack the molecular chaperone H2M, which is thought to be required for peptide loading onto MHCII. Future studies will directly assess the role of MHCII on AECIIs during *in vivo* flu infections.

P60. "RIPK1 kinase activity is critical for control of *Yersinia*"

Meghan Wynosky-Dolfi, Daniel Sorobetea, Mathieu Bertrand, Igor Brodsky

Cell death is a critical function in tissue differentiation and response to cellular damage or infection. RIPK1 serves as a central hub for cell fate decisions. RIPK1 scaffolding function can lead to prosurvival and proinflammatory gene expression, whereas RIPK1 kinase activity can be activated leading to cell death. We have recently shown that mice lacking

the kinase activity of RIPK1 fail to control *Yersinia* infection. These mice lack the necessary cell death signals that RIPK1 provides during infection, and therefore succumb to *Yersinia*. These two arms of RIPK1 activity have become appreciated in recent years, but the switch between these pathways remains poorly understood. RIPK1 can be phosphorylated, but the sites and what kinases phosphorylated RIPK1 remained unclear. We have identified a phosphorylation site on Ser25 by IKK. This phosphorylation site serves as a key modification that inhibits RIPK1 kinase activity and prevents death induced by *Yersinia* infection and TNF- or LPS-mediated RIPK1-dependent cell death. By mimicking Ser25 phosphorylation, we can protect cells from RIPK1-dependent cell death. Importantly, mimicking this Ser25 phosphorylation did not protect mice from *Yersinia* infection further demonstrating that RIPK1-induced cell death serves an important function in the control of *Yersinia* infection.

P61. "Role of FBXW7 isoforms in normal and leukemic human B cells"

Scarlett Y. Yang, Katharina E. Hayer, Vinodh Pillai, Andrei Thomas-Tikhonenko

Traditionally, B cell development is thought to be orchestrated by expression of various transcription factors (TF). However, recent developments indicate that the underlying mechanisms are more complex and might involve function of transcript variants. For example, E2A is a TF with two isoforms that regulates early B cell differentiation and class switching. The extent to which transcript variants contribute to human B cell development, humoral response, and neoplastic transformation remains unclear. To examine this, I begun three lines of research: 1. To isolate various progenitor and B cell subsets (early, pro, pre, immature) from bone marrow (BM). 2. To isolate malignant B cells from primary B-Cell Acute Lymphoblastic Leukemia (B-ALL) patient samples. 3. To isolate mature B cell subsets from tonsils and activate them via BCR ligation. All three approaches were followed by deep RNA-Seq and analysis with MAJIQ algorithm to identify transcript variants. Among thousands of genes expressing alternative transcripts in all three experimental settings, one gene common for all three sets is the F-box and WD Repeat Domain Containing 7 (FBXW7). FBXW7 encodes an E3 ubiquitin ligase that targets substrates to proteasomal degradation. Three coding isoforms of FBXW7 -- alpha, beta, and gamma -- differ in their subcellular localization. Although others have found that FBXW7 alpha could either promote or suppress cancer in different cellular contexts, role of FBXW7 beta remains unknown in normal and malignant B cells. My findings suggest: 1. The transition from early progenitor to pro-B is accompanied by the alpha-to-beta isoform switch, which is then reversed at later stages. 2. B-ALL samples from Children's Hospital of Philadelphia and St. Jude Children's Hospital exhibit differential FBXW7 alpha-to-beta ratios compared to normal counterparts. 3. Knockdown of FBXW7 alpha in a lymphoblastoid cell model results in modest accumulation of MYC protein. These results suggest that expression of FBXW7 isoforms are regulated throughout human B cell development, and FBXW7 regulates turnover of oncoproteins in malignant B cells. I hypothesize that various FBXW7 isoforms target distinct protein substrates for degradation and achieve temporal regulation of gene expression in B cell development in a physiological setting and can play a pathological role in neoplastic transformation. FBXW7 beta may target substrates distinct from that of alpha, thereby ensuring proper cellular functions at pro-B cell stage.

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COVER IMAGE // "Leukocytes swarm pancreatic cancer" is a stain of a pancreatic cancer tissue with PDL1 (brown), CD8 (purple), CK19 (teal) and Ki67 (yellow). Copyright Beatty Lab.

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