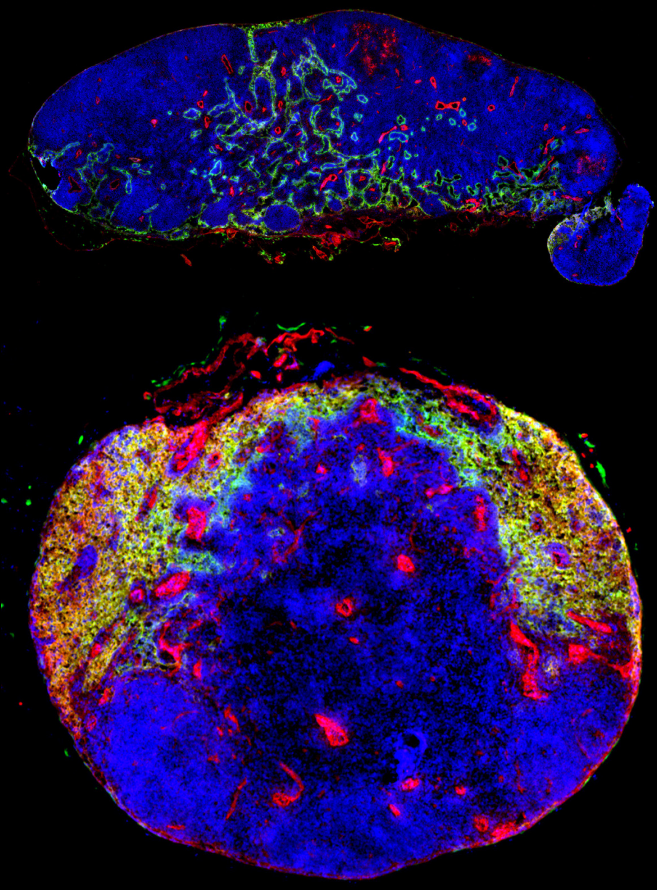


29TH ANNUAL IMMUNOLOGY GRADUATE GROUP RETREAT

OCTOBER 21 - 23
2016

ST. MICHAELS
MARYLAND

FRIDAY, OCTOBER 21

11:00 AM – 1:30 PM	**Registration** (Lobby)
12:00 – 1:20 PM	**Lunch** (Bayview Restaurant)
1:20 – 1:30 PM	**Welcome** (Prospect Bay Ballroom) Bruce Freedman, VMD, PhD – Associate Professor, Pathobiology, Veterinary Medicine Michael May, PhD – Associate Professor, Biomedical Sciences, Veterinary Medicine
1:30 – 3:10 PM	**Session I: Host-Pathogen Interactions** (Prospect Bay Ballroom) S1A. Daphne C. Avgousti, PhD "A Core Viral Protein Binds Host Nucleosomes To Sequester Immune Danger Signals" S1B. Katherine S. Forsyth "Antigen transfer is the major source of MHC-II restricted epitopes during poxvirus infection" S1C. Kelly Rome "Trib1 suppresses CD4 T cell activation and restricts the immune response to chronic infection" S1D. Sarah Sneed "Midgut commensals regulate Zika and Sindbis Virus Infection in Aedes aegypti mosquitoes" S1E. Ryan Staupe (session chair) "Regulation of effective vs. ineffective B cell responses to chronic viral infection"
3:10 – 3:30 PM	**Break**
3:30 – 5:10 PM	**Session II: Immune Regulation and Metabolism** (Prospect Bay Ballroom) S2A. Daniel Blumenthal, PhD "Modulation of T cell priming by dendritic cell stiffness" S2B. Christopher Ecker "Human T cell subsets differentially utilize fatty acid metabolism as a metabolic salvage pathway in response to low glucose" S2C. Daniela Gómez Atria, PhD "3D Imaging of bone marrow plasma cell niches" S2D. Christelle Harly, PhD "Tcf7 coordinates a transcriptional program underlying early innate lymphoid cell development that overlaps with the T cell developmental program" S2E. Jonathan DeLong (session chair) "A role for IL-27 in the regulation of Ly6C expression by T cells"
5:10 – 7:00 PM	**Room Check-in**
5:30 – 7:00 PM	**Dinner** (Bayview Restaurant)

FRIDAY, OCTOBER 21

7:00 – 8:10 PM	**Faculty Talks I** (Prospect Bay Ballroom) Montserrat Anguera, PhD Assistant Professor, Biomedical Sciences, School of Veterinary Medicine "Investigating the genetic basis for female-bias with autoimmunity: X-chromosome inactivation in immune cells" Michael Silverman, MD, PhD Assistant Professor, Pediatrics, Perelman School of Medicine "A protective MHC-II allele prevents autoimmunity by shaping the early-life microbiome"
8:10 PM – 12:00 AM	**Reception** (Duckblind Bar and Chesapeake Lounge)

SATURDAY, OCTOBER 22

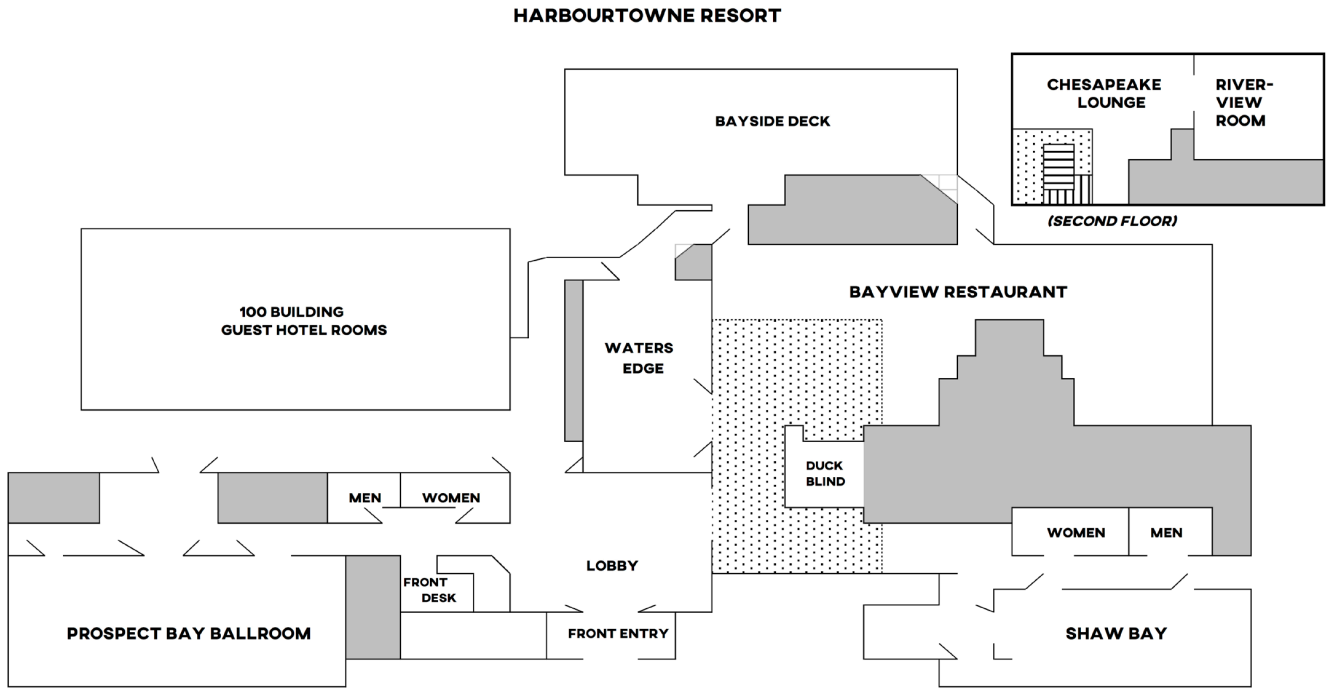
7:00 – 9:00 AM	**Breakfast Buffet** (Bayview Restaurant)
9:00 – 10:00 AM	**Session III: Immune-mediated Diseases** (Prospect Bay Ballroom) S3A. Jae Lee "A role for myeloid cells and hepatocytes in regulating metastasis in pancreatic carcinoma" S3B. Michael Leney-Greene "Mutations in GIMAP5 lead to a novel Mendelian disease of immune dysregulation" S3C. Awo Layman (session chair) "Ndfip1 limits mTORC1 activity in Regulatory T cells to prevent loss of stability and autoinflammatory disease"
10:00 – 11:10 AM	**Faculty Talks II** (Prospect Bay Ballroom) De'Broski Herbert, PhD Associate Professor, Pathobiology, School of Veterinary Medicine "Trefoil proteins: A troika for tissue regeneration" Gerald Linette, MD, PhD Chief Medical Officer, Cancer Immunotherapy; Clinical Director, Parker Institute "T cell repertoire diversity to melanoma neoantigens"
11:10 – 11:30 AM	**Break**
11:30 AM – 12:45 PM	**Keynote** (Prospect Bay Ballroom) Diane Mathis, PhD Professor of Microbiology and Immunobiology, Morton Grove-Rasmussen Chair of Immunohematology, Harvard Medical School "Immunological control of nonimmunological processes"
12:45 – 2:00 PM	**Lunch** (Bayview Restaurant) IGG 1st year PhD & 3rd year MD/PhD students meet in Riverview (above Bayview Restaurant)

SATURDAY, OCTOBER 22

2:00 – 3:00 PM	**Career Development for Trainees: Advice from Early-career and Transitioning Academic and Government Scientists** (Shaw Bay) Jorge Alvarez, PhD Assistant Professor, Pathobiology, School of Veterinary Medicine Montserrat Anguera, PhD Assistant Professor, Biomedical Sciences, School of Veterinary Medicine De'Broski Herbert, PhD Associate Professor, Pathobiology, School of Veterinary Medicine Vanja Lazarevic, PhD Investigator, Experimental Immunology Branch, Center for Cancer Research, NCI
3:00 – 5:30 PM	**Free Time**
5:30 – 7:00 PM	**Poster Session and Happy Hour** (Shaw Bay)
7:00 – 8:30 PM	**Dinner and Awards** (Bayview Restaurant)
8:30 PM – 12:00 AM	**Reception** (Duckblind Bar and Chesapeake Lounge)

SUNDAY, OCTOBER 23

7:00 – 10:00 AM	**Breakfast Buffet** (Bayview Restaurant)
11:00 AM	**Room Checkout**



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S1A. "A Core Viral Protein Binds Host Nucleosomes To Sequester Immune Danger Signals"

Daphne S. Avgousti, Christin Herrmann, Katarzyna Kulej, Neha Pancholi, Nikolina Sekulic, Joana Petrescu, Rosalynn C. Molden, Andrew J. Paris, Philomena Ostapchuk, Patrick Hearing, G. Scott Worthen, Ben E. Black, Benjamin A. Garcia, Matthew D. Weitzman

Viral proteins mimic host protein structure and function to redirect cellular processes and subvert innate defenses. Small basic proteins compact and regulate both viral and cellular DNA genomes. Nucleosomes are the repeating units of cellular chromatin and play an important role in innate immune responses. Viral encoded core basic proteins compact viral genomes but their impact on host chromatin structure and function remains unexplored. Adenoviruses encode a highly basic protein called protein VII that resembles cellular histones. Although protein VII binds viral DNA and is incorporated with viral genomes into virus particles, it is unknown whether protein VII impacts cellular chromatin. Our observation that protein VII alters cellular chromatin led us to hypothesize that this impacts antiviral responses during adenovirus infection. We found that protein VII forms complexes with nucleosomes and limits DNA accessibility. We identified post-translational modifications on protein VII that are responsible for chromatin localization. Furthermore, proteomic analysis demonstrated that protein VII is sufficient to alter protein composition of host chromatin. We found that protein VII is necessary and sufficient for retention in chromatin of members of the high-mobility group protein B family (HMGB1, HMGB2, and HMGB3). HMGB1 is actively released in response to inflammatory stimuli and functions as a danger signal to activate immune responses. We showed that protein VII can directly bind HMGB1 *in vitro* and further demonstrated that protein VII expression in mouse lungs is sufficient to decrease inflammation-induced HMGB1 content and neutrophil recruitment in the bronchoalveolar lavage fluid. Together our *in vitro* and *in vivo* results show that protein VII sequesters HMGB1 and can prevent its release. This study uncovers a viral strategy in which nucleosome binding is exploited to control extracellular immune signaling.

S1B. "Antigen transfer is the major source of MHC-II restricted epitopes during poxvirus infection"

Katherine S. Forsyth, Laurence C. Eisenlohr

Orthopoxviruses encode a number of immunomodulatory proteins that profoundly interfere with many aspects of the immune system. Ectromelia (ECTV), or mousepox, 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. While there are numerous examples of orthopoxvirus-encoded factors that interfere with both the innate immune system and MHC-I mediated CD8+ T cell activation, viral interference with MHC-II mediated CD4+ T cell activation is much less well characterized. Here we show that ECTV infection of antigen presenting cells (APC) inhibits their ability to activate CD4+ T cells directly. However, the mechanism of inhibition is not straight forward as neither dramatic down-regulation of surface MHC-II nor soluble factors are responsible. Despite this profound inhibition, CD4+ T cells are robustly activated *in vivo* in response to ECTV infection. The explanation that we have uncovered appears to be transfer of antigenic material from infected cells to uninfected APCs, reminiscent of class I-restricted cross-presentation. Furthermore, this mechanism is dependent in large part on the H2-M chaperone and is independent of the recycling pathway of MHC-II antigen presentation. Lastly, we show that the antigenic material transferred in large part does not require direct cell-cell contact and can pass through a 0.4 micron filter. Together these data show that ECTV can inhibit MHC-II mediated CD4+ T cell activation in addition to its many other immunomodulatory capabilities and that the host immune system has developed a mechanism of circumvention.

S1C. "Trib1 suppresses CD4 T cell activation and restricts the immune response to chronic infection"

Kelly Rome, Sarah Stein, Ethan Mack, Makoto Kurachi, John Wherry, Martha Jordan, Warren Pear

T cells are pivotal in the anti-viral immune response, however, over the course of a persistent infection T cells lose effector function and ultimately become exhausted leading to sustained viral load and chronic disease. CD4 T cells provide "CD4 help" to protect against CD8 exhaustion and promote the effector function of CD8 T cells and B cells. As a result, CD4 T cells have emerged as a promising new therapeutic target to bolster multiple arms of the anti-viral response, however, the mechanisms by which CD4 T cells orchestrate these responses are not fully understood. We identified the pseudokinase Trib1 as a negative regulator of CD4 T cell activation. Trib1 expression is significantly induced in activated CD4 T cells. Using CD4-cre^{+} Trib1$^{F/F}$ mice we demonstrated that Trib1-deficient CD4 T cells produce more IL-2 and proliferate more rapidly in response to stimulation. We employed the LCMV mouse model to assess the role of Trib1 in regulating the T cell response to viral infection. Trib1 expression is significantly elevated in CD4 T cells following LCMV infection in mice. CD4-cre^{+} Trib1$^{F/F}$ mice infected with a chronic strain of LCMV are more resistant to chronic infection as characterized by expanded T cell effector populations, enhanced T cell effector function, increased antibody production and decreased viral titers. Together, these results identify Trib1 as a negative regulator of T cell activation and a promising target for reinvigorating the T cell response during chronic infection.

S1D. "Midgut commensals regulate Zika and Sindbis Virus Infection in *Aedes aegypti* mosquitoes"

Sarah Sneed, Michael Povelones

In recent years, mosquito-borne viruses such as Dengue (DENV), Chikungunya (CHIKV), and Zika (ZIKV) have become globally disseminated and are a significant cause of human morbidity and mortality. In order for transmission to occur, viruses must first infect the mosquito midgut and subsequently pass into the hemolymph where they can circulate to infect other tissues. Numerous studies have demonstrated that the mosquito midgut is an important barrier that bloodmeal-acquired viruses must overcome. Therefore, understanding the mechanisms underlying successful infection of the midgut is crucial. The mosquito microbiome plays an important role in shaping the midgut environment and has been previously shown to regulate susceptibility to infection by different arboviruses. To contribute to this body of knowledge, we have tested whether reducing midgut commensal bacteria affects *Ae. aegypti* infection by Zika and Sindbis (SINV) viruses. Our preliminary data suggests that depletion of commensals by antibiotics prior to viral infection results in a decrease in viral transcript levels. We are validating these results by assaying virus at the protein level by western blot and determining localization by confocal microscopy. Specifically, we are interested in exploring the mechanism behind this phenotype. 16S sequencing and CFU plating will be used to define the bacterial signals that elicit infection-permissive responses from the intestinal epithelium. Toll and Imd NF-κB signaling pathways are important for regulation of the gut microbiome. Bacterial products bind to cell surface receptors and activate signaling in the intestinal epithelium. Therefore, we are using RNA-seq to characterize the differences between antibiotic and sugarfed *Ae. aegypti* in activation of these pathways during viral infection. Results from these studies will lead to mechanistic insights that may yield novel strategies to block disease transmission.

S1E. "Regulation of effective vs. ineffective B cell responses to chronic viral infectionEndFragment"

Ryan P. Staupe, E. John Wherry

Human immunodeficiency virus (HIV) and hepatitis C virus (HCV) are chronic viral infections that cause significant morbidity and mortality worldwide. Current efforts to vaccinate against these pathogens have been unsuccessful and have failed to induce effective neutralizing antibodies capable of mediating sterilizing immunity. Natural HIV or HCV infection elicits the production of affinity matured class-switched antibodies that target viral proteins. While these antibodies can drive viral evolution, they are ineffective at controlling infection. Moreover, it is now clear that the evolution of these antibody responses, and the B cells that produce them during chronic infection are radically different than those generated by acute infection or vaccination. Despite these observations, little is

known about the underlying mechanisms of B cell differentiation and control of effective antibody production during chronic viral infection. We have developed a novel technique that allows us to track the virus-specific B cell response in the lymphocytic choriomeningitis virus (LCMV) model, a mouse model of chronic viral infection. Using this technique, we have found that chronic viral infection does not cause a skewing of the virus-specific B cell response to the plasma cell fate and away from the germinal center response but induces a robust plasma cell response in an antigen-level and T cell-dependent manner. These results suggest that alter differentiation of virus-specific B cells early during the response to chronic viral infection may contribute to the temporal delay in development of effective neutralizing antibody responses during chronic infection.

S2A. "Modulation of T cell priming by dendritic cell stiffness"

Daniel Blumenthal, Janis Burkhardt

Priming of T-cell responses by dendritic cells (DCs) is essential for protective immunity to pathogen invasion and cancer. T cell activation requires intimate cell-cell interactions at a site termed the immunological synapse (IS), and mounting evidence from a variety of sources indicates that this process involves mechanotransduction. Although events at the IS have been extensively studied on the T-cell side, much less is known about the role DC biology plays in this interaction. One of the most remarkable skills of DCs is their ability to alter their biophysical properties (mobility, stiffness, contractility etc.) to carry out specific functions during the course of an immune response. Therefore, we suggest that the biophysical properties of the DC surface represent an unexplored control point for T cell priming. Using atomic force microscopy (AFM), we show that upon LPS- induced maturation, DC stiffness increases from ~2kPa to ~4kPa in an actin- cytoskeleton controlled process. We show that several actin regulatory pathways downstream of Rho GTPases Rho1 and Rac/CDC42 take part in DC stiffness modulation. Interestingly, activating T-cells with substrates of different compliances show a threshold for activation at a range of 1-4 kPa, similar to the range over which DC stiffness changes during LPS-induced maturation. Finally, using DCs derived from the bone marrow of several KO mice, we show a correlation between the ability of mature DCs to prime T-cells and a defect in mature DC stiffness.

S2B. "Human T cell subsets differentially utilize fatty acid metabolism as a metabolic salvage pathway in response to low glucose"

Christopher Ecker, James Riley

T cells play a crucial role in many anti-tumor responses *in vivo*. The success rate of adoptive T cell therapies has been modest in solid tumors in which cancer cells set up an immunosuppressive niche. We speculate that the low glucose conditions present in many solid tumor microenvironments contribute to the immune dysfunction observed. Previous work established that in the absence of glucose, T cells are impaired in their inability to proliferate and produce IFN_{γ}. We sought to understand the physiological pathways that T cells utilize when placed in low glucose. Furthermore, we examined how younger naïve T cells, central memory (CM) cells and more terminally differentiated effector memory (EM) cells adapted uniquely to low glucose. Naïve and CM cells rely heavily on fatty acid metabolism to increase oxidative phosphorylation to efficiently expand in low glucose. However even though these cells survive by increasing exogenous and intrinsic sources of fatty acids, these cells produce much lower IFN_{γ} in low glucose. EM cells are unable to increase oxidative phosphorylation, rely less on both fatty acid synthesis and fatty acids from exogenous sources to survive but still can maintain IFN_{γ} expression. By understanding the endogenous salvage pathways that T cells utilize in the tumor microenvironment, we can enhance their ability to adapt, survive, and function for future immunotherapy in solid tumors.

S2C. "3D Imaging of bone marrow plasma cell niches"

Daniela Gómez Atria, Tony E. Smith, David Allman

Plasma cells (PCs) are essential for establishing and maintaining antibody-mediated immunity. Once generated, many PCs migrate to the bone marrow (BM) where it is believed they localize in specialized regulatory microenvironments, or "niches," where they must remain indefinitely to gain continuous access to key survival signals. However, little is known about these unique microenvironments or how plasma cell niches are established. We have visualized the 3-dimensional BM PC niche using confocal microscopy imaging coupled with bone/tissue clearing in conjunction with reporter mice and relevant fluorescent antibodies. Moreover, by assigning XYZ coordinates for every cell together with nearest-neighbor analyses, we have identified significantly non-random associations between PCs and another cell types and structures throughout the BM. The majority of PCs localize in perivascular niches, distant to bone-proximal regions, usually in discrete clusters in association with hematopoietic stem cells (HSCs) and CXCL12-producing stromal cells. Moreover, both immature short-lived and mature long-lived PCs were found within perivascular clusters. These results contrast for mature recirculating B cells, which localized preferentially near bone-rich regions. We propose that HSCs and PCs utilize similar sources of CXCL12 and other elements required for their survival. We further propose that our results reveal distinct BM regions wherein immobile HSCs and plasma cells are spatially segregated from mobile hematopoietic cells.

S2D. "*Tcf7* coordinates a transcriptional program underlying early innate lymphoid cell development that overlaps with the T cell developmental program"

Christelle Harly, Maggie Cam, Jonathan Kaye, Hai-Hui Xue, Avinash Bhandoola

Innate lymphoid cells (ILC) and T cells have very similar effector functions, controlled by overlapping transcriptional programs. Little is known about the early steps of development of ILC compared to T cells, and it is particularly unclear how these two lineages diverge from each other while keeping access to similar effector programs. Here, we use transcriptional profiling to provide candidate regulators of early ILC specification and commitment. We identify a transcriptional program shared between T cells and ILC at the earliest steps of development that includes the transcription factors *Tox*, *Tcf7*, *Gata3*, and *Bcl11b*. We find that, similarly to its role during T cell development, *Tcf7* is required during ILC development for proper expression of *Gata3*, *Bcl11b* and many structural genes including genes of the TCR signaling pathway. Our study provides a basis for understanding similarities in T cell and ILC differentiation and effector function.

S2E. "A role for IL-27 in the regulation of Ly6C expression by T cells"

Jonathan DeLong, Aisling O'Hara Hall, Gaia Muallem, Christopher A. Hunter

Ly6C is a GPI-anchored glycoprotein expressed on the surface of several hematopoietic cell lineages. On T cells, its expression distinguishes short-lived effector CD4+ T cells from memory precursor effector cells. The function of Ly6C is unclear but it is associated with homing to lymph nodes and limiting T cell activation. We are investigating the factors that regulate the expression of Ly6C and their possible roles in infection. We found that TCR stimulation is not sufficient to induce Ly6C expression, but that the induction of Ly6C requires TCR stimulation in combination with IL-27, interferon alpha, or interferon-gamma. Each of these cytokines signals through STAT1 and T-bet, but these signaling molecules were not found to be involved in the induction of Ly6C. The *in vivo* significance of cytokine-mediated Ly6C induction is seen during toxoplasmosis, when both naïve and activated parasite specific effector CD4+ and CD8+ T cells upregulate Ly6C expression. This upregulation is reduced in mice that lack IL-27. While these results suggest that cytokines have a major role in the regulation of Ly6C expression, it remains unclear how Ly6C affects T cell function and what role cytokine-mediated expression might play in the specific expression of Ly6C on effector but not memory populations.

S3A. "A Role for Myeloid Cells and Hepatocytes in Regulating Metastasis in Pancreatic Carcinoma"

Jae Lee, Paige M. Porrett, Whitney L. Gladney, Gregory L. Beatty

Pancreatic ductal adenocarcinoma (PDAC) is the fourth-leading cause of cancer-related deaths in the United States with metastasis as the major cause of mortality. The vast majority of PDAC patients present with metastatic disease with the liver representing the most common site of disease spread. While the propensity of PDAC to metastasize to the liver may reflect mechanical trapping of tumor cells shedding from the portal circulation, primary PDAC cells have also been suggested to secrete factors that may precondition the liver for metastasis. In this study, we have investigated the impact of PDAC development on liver biology using the *LSL-Kras*$^{G12D/+}$;*LSL-Trp53*$^{R172H/+}$;*Pdx-1-Cre* (KPC) mouse model of PDAC. We found that KPC mice have an increased susceptibility to tumor seeding in the liver, even before they display histological evidence of invasive PDAC. To examine this increased propensity for tumor seeding in the liver, we investigated for changes in cellular and stromal architecture in the liver of KPC mice versus control mice. We found that F4/80^{+} myeloid cells accumulated within the liver of KPC mice prior to the development of invasive PDAC. In addition, these myeloid cells demonstrated activation of Signal Transducer and Activator of Transcription 3 (STAT3) signaling. Similar to myeloid cells, hepatocytes also displayed activation of STAT3 signaling. Extracellular matrix proteins, including type I collagen and fibronectin, were increased within the liver as well. In order to determine the role of tumor cells in driving cellular and matrix changes seen in the liver microenvironment, we established intraperitoneal and orthotopic models of PDAC. Using these models, we found that implantation of pancreatic tumor cells led to an increased number of STAT3-activated myeloid cells and hepatocytes and fibrosis in the liver. In addition, we performed parabiosis of tumor-implanted mice to wildtype mice to determine the systemic nature of this response and found similar changes in the liver of both groups of mice. These results suggest that tumor cells may systemically secrete factors that activate STAT3 signaling. In support of this hypothesis, we have observed that pre-invasive tumor lesions produce interleukin-6 (IL-6), a cytokine that can activate STAT3 signaling, *in vivo*. Antibody blockade of IL-6 receptor after tumor implantation reduced the number of STAT3-activated myeloid cells and hepatocytes in the liver. Together, our findings suggest a role for IL-6 produced during PDAC development in driving a pro-metastatic niche in the liver.

S3B. "Mutations in GIMAP5 lead to a novel Mendelian disease of immune dysregulation"

Michael Leney-Greene, Michael Lenardo, Helen Su

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 GIMAP5 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 these GTPases in the immune system remains a fundamental gap in our knowledge. Due to extensive similarity in phenotypes between mice with T cell specific deletions of critical autophagy genes and GIMAP5 deficient mice, we hypothesized that GIMAP5 deficient mice may have defects in this pathway. We observed constitutively increased levels of LC3-II in GIMAP5 deficient T cells at baseline, but overall decreased flux through the pathway consistent with a defect in autophagosome maturation or processing. Furthermore, we observed that GIMAP5 co-immunoprecipitates and colocalizes with Rab7 which has previously been shown to be required for autophagosome maturation and lysosomal fusion. Overall, our data suggest that GIMAP5 is a novel regulator of autophagy and rab7 activity specifically in lymphocytes.

S3C. “Ndfip1 limits mTORC1 activity in Regulatory T cells to prevent loss of stability and autoinflammatory disease”

Awo Akosua Kesewa Layman, Guoping Deng, Claire E. O’Leary, Samuel Tadros, Rajan Thomas, Emily Moser, Andrew Wells, Nicolai Doliba, Paula M. Oliver

Foxp3+ T regulatory (T_{reg}) cells keep the immune system in check while allowing robust anti-pathogen responses. Here, we show that Ndfip1, a co-activator of Nedd4-family E3 ubiquitin ligases, is required for T_{reg} cell function. In Ndfip1-deficient T_{reg} cells, stability and function was compromised, resulting in inflammation at barrier surfaces. Loss of Ndfip1 resulted in the expansion of CD44+ T_{reg} cells that were highly proliferative, prone to produce IL-4, and more likely to lose Foxp3 and convert into IL-4 producing Th2 cells. To understand the mechanistic basis for these changes, we analyzed the proteomes of Ndfip1-sufficient or -deficient CD44+ T_{reg} cells, and found that Ndfip1-deficient T_{reg} cells exhibited increased metabolic activity. Based on these and other data, we propose that Ndfip1 controls TORC1 activity in T_{reg} cells to adjust glycolytic rate and preserve lineage stability.

P1. "Reprogramming of exhausted T cells following cure of chronic viral infection"

Mohamed Abdel-Hakeem, E. John Wherry

T-cell exhaustion is a hallmark of immune failure to control cancer and chronic infection. Blocking inhibitory receptors (e.g. PD-1) can re-invigorate exhausted T cells (Tex), but most patients fail to achieve tumor control. Thus, deeper understanding of other molecular and epigenetic mechanisms underlying reversal of T-cell exhaustion is needed. Treatment of chronic HCV with novel antivirals leads to complete cure. Whether Tex in cured subjects become "reprogrammed" into more functional effector or memory T cells (Tmem) is unknown.

To address this, we use the LCMV mouse model, where we achieved "cure" by adoptive transfer of Tex from chronically-infected mice to immune mice. We aim to determine the cellular profiles, epigenetic signatures, and population dynamics of virus- specific Tex.

Our data indicate that some markers of Tmem may be recovered, while other aspects are not corrected simply by eliminating antigen exposure. We are currently investigating whether this is linked to selective recovery of a specific subset of Tex, or to changes in the epigenetic landscape.

This study would enhance our understanding of the molecular signatures of recovery of Tex, that could expose novel therapeutic targets to reverse immune- exhaustion.

P2. "Modulation of the neuroblastoma microenvironment by polyamine blockade"

Adriana D. Benavides, Annette Vu, Gabrielle M. Ferry, Michael D. Hogarty, Hamid Bassiri

High-risk neuroblastoma (NB), which accounts for a considerable portion of pediatric cancer-related mortalities, results from *MYCN*-amplification and alterations in Myc-regulated pathways. Despite improvements in therapy, long-term survival rates remain poor. NBs have elevated polyamine (PA) levels due to Myc's targeting of ODC, the rate-limiting enzyme for PA synthesis. It has been shown that PA blockade using the drug DFMO leads to a greater reduction in NB growth *in vivo* than *in vitro*, suggesting a tumor-cell extrinsic effect. PAs can drive the differentiation of immune suppressive cells, while blockade can increase tumor-infiltrating leukocytes (TILs). However, previous studies investigating effects of DFMO on the TME are incomplete. Therefore, we sought to characterize the TME in a spontaneous *MYCN*-driven NB mouse model with and without DFMO. The frequencies of various TILs were assessed. We observed that DFMO alters the NB TME by increasing frequencies of DCs and NK cells while maintaining G-MDSCs. These data support our hypothesis that PA blockade induces distinct immune changes in the TME that could allow for a more efficient anti-tumor response.

P3. "Profiling T cell exhaustion by mass cytometry"

Bertram Bengsch, E. John Wherry

In the response to chronic infection and cancer, hypofunctional "exhausted" T cells accumulate, that can express multiple inhibitory receptors and exhibit major changes in transcriptional and differentiation programming. Targeting exhausted T cells by inhibitory receptor blockade is a key element of novel immunomodulatory blockades. Despite these insights, phenotypic analysis of exhausted cells remains challenging since many pathways operative in exhausted cells may also operate in other subsets; in addition, heterogeneity of exhausted cells is beginning to be recognized. We address these issues by high-dimensional single-cell phenotyping by mass cytometry in HIV and cancer, generating a comprehensive exhaustion landscape suitable to dissect the heterogeneity of exhausted T cells. Using dimension-reduction and clustering approaches, we identify different subsets with altered co-expression of inhibitory receptors, metabolic markers, differentiation markers and memory-like phenotypes. We identify clear differences between exhausted subsets in chronic infection and cancer. These results have implications for our understanding of the immunobiology of exhausted T cells and provide insights relevant for viral infections and cancer.

gastrointestinal flora is required for maximal TLR9 responsiveness and this translates into decreased TLR9 mediated cytokine storm in the presence of antibiotics. Further studies are targeted at elucidating the mechanism by which gut-flora promotes normal TLR9 responsiveness. Ultimately, these studies might provide an explanation for the "immunomodulatory" effect that has been attributed to various antibiotics in the clinical literature.

P4. "Macrophages in Secondary Lymphoid Organs Regulate T cell Tolerance and Immunosurveillance in Pancreatic Carcinoma"

Fee Bengsch, Anni Liu, Kathleen Graham, Gregory L Beatty

Pancreatic Ductal Adenocarcinoma (PDAC) remains a deadly disease with very limited treatment options. Immune therapy approachesin PDAC are exciting avenues but have been of limited success, which may be due to the induction of peripheral tolerance and low numbers of tumor-infiltrating T cells. We hypothesize that successful immunotherapy in PDAC will require strategies that break T cell tolerance. In the KPC mouse model of PDAC, we previously showed that macrophages regulate the capacity of chemotherapy in combination with a CD40 agonist (FGK45) to induce T cell anti-tumor immunity. Specifically, we found that peripheral phagocytes which can be depleted using clodronate-encapsulated liposomes (CEL) are critical regulators of T cell tolerance. In this study, we investigate the mechanism by which CEL-depleted phagocyte regulate tolerance. We find that CEL depletes macrophages in the spleen and abdominal lymph nodes, reversings the pathologic accumulation of macrophages in secondary lymphoid organs draining from a PDAC tumor. CEL depletes CD11b+CD169+F4/80- subcapsular sinus macrophages (SSM) and CD11b+CD169+F4/80+ medullary sinus macrophages (MSM). These macrophage populations are exposed to tumor-derived antigen and apoptotic cell vesicles entering the lymph node, and have been implicated as central orchestrators of tolerance in autoimmune models. Consistently, we found that depletion of these first-line phagocytes leads to a redistribution of apoptotic cell vesicle uptake in the lymph node. In sum, our findings suggest that tumor-derived antigens are sequestered by tolerogenic macrophages in peri-tumoral lymph nodes impairing optimal induction of T cell responses. Thus, strategies aiming to disrupt the tolerogenic functions of these macrophages may represent a novel approach to enhance immune responses against PDAC.

P5. "Defining the Role of BinCARD in Programmed Cell Death"

Elisabet Bjanes, Igor Brodsky

Regulated cell death is a conserved immune mechanism that contributes to the clearance of bacterial pathogens. Apoptosis and pyroptosis are caspase-dependent forms of regulated cell death induced by enteric bacterial pathogens, including *Yersinia pseudotuberculosis* and *Salmonella* Typhimurium, respectively. However, the mechanism by which the innate immune system determines cell fate following infection remains poorly understood. Apoptosis and pyroptosis are regulated by a family of related proteins that contain homotypic protein-protein interaction domains of the caspase recruitment (CARD) and death domain (DD) families. Here, we describe a new regulator of caspase-dependent death in bone marrow-derived macrophages (BMDMs) known as CARD19, or Bcl10-interacting protein with CARD (BinCARD). We demonstrate that BinCARD-deficient BMDMs have substantial defects in caspase-dependent cell death in response to multiple stimuli including infection by *Salmonella* Typhimurium, *Yersinia pseudotuberculosis*, or treatment with the broad-spectrum protein kinase inhibitor staurosporine. We also show that BinCARD localizes to the mitochondria, suggesting a potential role in mitochondrial stability or regulation of membrane potential. BinCARD is closely related to another mitochondrial CARD-containing protein, MAVS (mitochondrial antiviral signaling protein), which forms a signaling scaffold that initiates interferon responses to viral pathogens. This suggests that BinCARD could modulate cellular responses to bacterial and apoptotic-associated stimuli via a similar mechanism to MAVS. Future studies will dissect the mechanism by which BinCARD contributes to cell death and innate host defense against microbial infection.

P6. "Hemophagocytic Lymphohistiocytosis can occur independently of IFNγ in a murine model"

Thomas Burn, Julia Elizabeth Rood, Lehn Weaver, Portia A. Kreiger, Edward M. Behrens

Hemophagocytic lymphohistiocytosis (HLH) is a cytokine storm disease caused by a systemic hyperinflammatory response that leads to severe hypercytokinemia, hepatosplenomegaly, multiple cytopenias, and ultimately death. Familial forms of the disease have been traced to deficiencies in the killing capability of cytotoxic lymphocytes resulting from mutations in the genes encoding perforin or molecules required for cytolytic granule release. Infection of perforin knockout mice (PKO) with an acute strain of lymphocytic choriomeningitis virus (LCMV) mimics HLH disease, and studies using these mice implicate excessive production of IFNγ by CD8+ T- cells as the driver of disease, prompting clinical trials aimed at targeting this cytokine. However, recent evidence from humans has suggested that HLH is not absolutely IFNγ-dependent, and to test the hypothesis that HLH can develop in the absence of IFNγ altogether, we bred an IFNγ/perforin double knockout mouse (DKO). We show that LCMV infection of DKO mice leads to rapidly fatal disease, and these mice fulfill many of the clinical diagnostic criteria for HLH disease. The qualitative nature of disease in DKO mice does however differ from disease in PKO mice and is characterized by extensive neutrophilia, and an altered inflammatory cytokine profile dominated by IL-6 and IL-1β. Surprisingly, DKO mice exhibited equivalent expansion of LCMV-specific CD8+ T-cells, and their role in disease pathogenesis will be a focus of future studies. We therefore describe a novel model of HLH, and show that this disease can manifest in the absence of IFNγ prompting an adjustment to the current dogma.

P7. "Single cell gene expression profiling identifies population dynamics during T cell exhaustion"

Zeyu Chen, Zhicheng, Laura Vella, Ramin Herati, Bertram Bengsch, Erietta Stelekati, Hongkai Ji, E. John Wherry

CD8+ T cells play a key role in antiviral and antitumor responses. However, during chronic infections and cancer, CD8+ T cells become dysfunctional and fail to form functional memory, a process described as "exhaustion". Since exhaustion is a constitutively stressful process for T cells, one of the key questions in the field is how to map cell fates during exhaustion, and identify the subpopulations that have the capacity to be manipulated in vivo for better clinical outcome.

In this study, we used acute and chronic *Lymphocytic Choriomeningitis virus (LCMV)* infections to trigger either memory or exhausted T cell generation, sorted single antigen specific CD8+ T cells and performed single cell multiplex qPCR to profile transcriptional features. First, we found that even at the early stage of chronic infection, the population dynamics was more complex than acute infection, and we identified a unique "early exhausted" population (T_{eEx}) in the chronic infection setting, which showed not only certain exhausted features, but also multiple over-activated signature. Interestingly, when we used pseudo-Time reconstruction in Single-Cell RNA-seq Analysis (TSCAN) to digitally map the cell fate during T cell exhaustion, the T_{eEx} was not predicted to generate the clinically observed late exhausted T cells (T_{Ex}), which indicated that the over-activated cells might have short lifespan and T_{Ex} might come from the less activated but more persisting population. Using surface markers, we identified T_{eEx} as KLRG1+CD39+CD200R+. With lineage tracing experiments, we found a significant short lifespan of T_{eEx} during exhaustion process comparing to other populations. Further TSCAN analysis showed that reactivating T_{Ex} with antiPD-L1 treatment reinvigorated T_{Ex} into effector-like cell state but not T_{eEx} state, suggesting that T_{eEx} is a terminal T cell state at early stages during chronic infection, which could tolerate high levels of antigen stimulation. Together, we found a T_{eEx} population, which showed a unique cell fate during T cell exhaustion, and our findings also suggested that T cells persisting during the late exhaustion phase are derived from the less activated T cells during early infection phase.

P8. "Astrocytes modulate the migratory capacity of regulatory T cells during neuroinflammation"

Lara Cheslow, Jorge Ivan Alvarez

The infiltration of T cells into the central nervous system (CNS) is a pivotal step in the development and progression of neuroinflammatory diseases like multiple sclerosis (MS) and its animal model experimental autoimmune encephalitis (EAE). As major mitigators of immune responses, regulatory T cells (Tregs) are directly

implicated in modulating the severity of EAE, though their suppressive abilities seem reduced in MS patients. Past studies making use of human in vitro models have shown that Tregs migrate more easily through the CNS endothelium than their conventional counterparts. However, we have found that unlike inflammatory CD4 T cells, Tregs are absent from the inflamed CNS parenchyma and are mostly concentrated within the meningeal microenvironment. Our studies seek to explain this paradox and implicate the barrier formed by the glia limitans juxtaposing the meningeal compartment in selectively preventing the migration of Tregs into the brain parenchyma. We have found that human and mouse Treg cells migrate significantly less across the glia limitans than conventional CD4 T cells. Currently, we are investigating the impact that astrocyte-derived Sonic hedgehog (Shh), a neuroprotectant that reduces neuroinflammation and promote neurovascular integrity, may have on Treg migration. Our findings hold potential for discovering an important role for Shh in the migration of Tregs across the glia limitans and immune regulatory mechanisms within the meningeal/perivascular microenviroment.

P9. "Discovering transcriptional controllers of thymic epithelial cell development"

Jennifer Cowan, Maggie Cam, Avinash Bhandoola

The specialized microenvironment of the thymus is essential for T cell development and repertoire selection. Thymic epithelial cells (TEC) are an essential part of this microenvironment, yet little is known about TEC differentiation and maintenance. TEC progenitors remain poorly identified and progenitor-successor relationships unclear. However, the proliferative and engraftment potential of TEC have been reported to differ greatly at distinctive developmental stages in mouse. Fetal TEC have the capacity to undergo successful engraftment following intrathymic injection into adult hosts, a characteristic not shared by their adult counterparts. This alteration to engraftment potential with increasing age may be a consequence of diverse molecular controls of the TEC subsets at different stages in development. Identification of differentially expressed transcriptional controllers of fetal and adult TEC may offer an insight into the drivers of the functional differences observed. We, therefore, are exploring the genetic signature of isolated TEC subsets at different stages in development using RNA sequencing analysis. We sequenced cortical and medullary TEC, isolated between embryonic day 13.5 and 4 weeks. The comparison of transcriptomes from fetal and adult TEC may reveal new genes controlling TEC properties throughout development.

P10. "Dissecting the small intestine niche for regulatory T cells"

Elisa Cruz-Morales, Terri Laufer

Foxp3+ regulatory T cells (Tregs) are required for the maintenance of intestinal homeostasis. They differentiate in the thymus (natural or nTregs) or are generated from conventional T cells in the periphery (named inducible T regs). Past studies have suggested that the repertoire of Tregs in the intestinal lamina propria (LP) is skewed towards iTregs with specificity for food antigens and commensal bacteria. However, using a transgenic mouse model, that lacks peripheral TCR-MHCII interactions and generation of extra-thymic Tregs (K14), our laboratory has previously demonstrated that the intestinal lamina propria is a unique niche for Tregs that can be filled and maintained independently of MHCII. We now present further cellular and molecular characterization of these regulatory T cells steered towards understanding the establishment of the intestinal Treg repertoire.

nTregs emerge from the thymus as quiescent “central” Tregs and can differentiate following TCR-dependent engagement in the periphery into “effector” Tregs that persist in non-lymphoid tissues. However, intestinal Tregs in K14 mice have an effector phenotype. Additionally, “effector” Tregs in the K14 and WT LP express equivalent levels of Nur77, IRF4, and many components of a TCR- dependent cassette of genes. These results suggest that there are local MHCII- independent pathways in the intestinal LP that can activate downstream TCR signaling and guide the differentiation and/or localization of effector Tregs. Indeed, short-term costimulation blockade with CTLA4Ig decreases the expression of Nur77 on intestinal Tregs of K14 mice.

The intestinal LP is rich in MHCII+ APCs able to interact with T cells through both cognate and non-cognate pathways. Confocal, 2-photon and CLARITY microscopy demonstrate that Tregs in K14 are in close contact with B

cells and DCs in the intestinal villi and also accumulate within B cell aggregates, suggesting that the tissue-specific characteristics of the LP environment permit Tregs and APCs to interact independently of specific antigenic signals. Therefore, it seems plausible that in the absence of MHCII, LP Tregs are still able to receive signals from APCs through cell-adhesion molecules, CD28, and cytokine stimulation to induce and maintain a local population of effector Tregs and suggest pathways that may be targeted for the antigen-independent expansion of local Treg populations.

P11. "Mechanism of caspase-8-dependent gene regulation"

Alexandra DeLaney, Igor Brodsky

Caspases regulate cell death programs in response to environmental stresses, including infection and inflammation, making their expression and function critical for the regulation of the mammalian immune system. Caspase-8 is necessary for production of inflammatory cytokines and host defense against infection by multiple pathogens including *Yersinia*, but whether this is due to death of infected cells or an intrinsic role of caspase-8 in TLR-induced gene expression is unknown. Whether caspase-8 activity is important for inflammatory gene expression during bacterial infection has not been investigated. Here we report that caspase-8 plays an essential cell-intrinsic role in innate inflammatory cytokine production *in vivo* during *Yersinia* infection. Moreover, caspase-8 enzymatic activity regulates gene expression in response to bacterial infection as well as TLR signaling independently of apoptosis. We have implicated the deubiquitinase CYLD as the downstream mediator of the caspase-8-dependent TLR signaling. This leads us to propose a model in which a non-apoptotic conformation of caspase-8 maintains catalytic activity that facilitates the cleavage and degradation of downstream mediators that negatively regulate gene expression in response to TLR activation. This work describes a mechanism for caspase-8-dependent host defense that is functionally and enzymatically distinct from its role in cell extrinsic apoptosis.

P12. "A systems biology approach to assessing changes in the T cell proteome brought about by TCR stimulation"

Joseph Dybas, Claire E. O'Leary, Paula M. Oliver

TCR stimulation triggers signaling pathways that direct T cell fate and function. Many such signals are transmitted via post-translational modifications such as ubiquitylation. However, the molecular targets of ubiquitylation are poorly understood. We have developed a combined genomics/proteomics strategy to quantify changes in cellular proteomes following TCR stimulation and to identify proteins that are ubiquitylated and/or degraded downstream of TCR triggering. We employed quantitative mass spectrometry to derive four proteomics datasets of resting and activated T cells: comparing 1) whole cell proteomes, 2) proteins that are ubiquitylated by either TUBE or 3) di-glycine remnant profiling, and 4) proteins that are degraded via the proteasome. In addition, RNAseq data was generated to determine changes in transcriptional regulation of these proteins. The overlap of these datasets allows for the identification of ubiquitylated substrates as well as the discrimination between those substrates that are directed for proteasomal degradation versus those that may have a non-degradative function. These data offer a systematic and quantitative view of how TCR stimulation regulates, and is regulated by, ubiquitin.

P13. "The role of Ndfip1 in macrophage-mediated bacterial clearance"

Natania Field, Emily Moser, Prasanna Chandramouleeswaran, Nikoff Vasquez, Ipsita Subudhi and Paula Oliver

Macrophages are essential components of the innate immune response to bacterial infections. While these cells must mount an appropriate inflammatory response to harmful pathogens, they require tight regulation to prevent host damage.

One way in which macrophages attenuate inflammatory signaling cascades is through ubiquitination. This post-translational modification can target a protein substrate for either downstream signaling or for degradation. E3 ubiquitin ligases are required to facilitate the formation of these chains. Nedd4-family interacting protein 1 (Ndfip1) activates multiple E3 ubiquitin ligases, including Itch, which is crucial for attenuating cytokine production by T cells. While Itch has been shown to limit macrophage activity, it is unclear how Ndfip1 functions in

macrophages, and how it affects macrophage antibacterial responses.

We have generated mice lacking Ndfip1 in myeloid cells (Ndfip1fl/flLysM-Cre). These mice have normal numbers of neutrophils, monocytes and macrophages in the lungs, peritoneum, and peripheral lymphoid organs. Upon i.n. infection with *Klebsiella pneumoniae*, WT mice begin to lose weight with bacterial loads peaking at day 3, but macrophages and neutrophils soon clear the bacteria. In contrast, mice that lack Ndfip1 in the myeloid lineage mount a more robust response and show reduced bacterial loads three days after infection. This result could be recapitulated when mice were infected via i.p. injection. We are currently exploring the mechanism through which Ndfip1 attenuates antibacterial responses, focusing primarily on the macrophage. This project will reveal new mechanisms used by myeloid lineage cells in eliminating pathogenic bacteria, providing insight into the regulatory mechanisms that prevent excess inflammation.

P14. "DNA double strand breaks suppress RAG expression and activity in a cell-type specific manner"

Megan Fisher, Craig Bassing

Adaptive immunity requires the production of lymphocytes capable of recognizing a broad and often unpredictable array of potential foreign antigens. In jawed vertebrates, B and T lymphocytes achieve the necessary diversity of antigen receptors (AgRs) by V(D)J recombination. The Rag1/Rag2 (RAG) endonuclease initiates V(D)J recombination by making DNA double-strand breaks (DSBs) at specific V, D, and J gene segments; DSB repair factors process RAG-generated DSBs to produce complete AgR genes. While RAG-induced DSBs are crucial for B and T cell development, the presence of AgR translocations in many human cancers and mouse models suggests that V(D)J recombination poses a threat to the genomic stability of developing lymphocytes. As RAG activity in the presence of DSBs elsewhere in the genome could increase the probability of translocation, I hypothesized that pre-existing DSBs would inhibit RAG activity. Consistent with this hypothesis, I have found that pro-B and pre-B cells rapidly suppress *Rag1* and *Rag2* mRNA expression after exposure to ionizing radiation (IR), which induces DSBs. CD4- CD8- double positive thymocytes also suppress *Rag1* and *Rag2* expression in response to DSBs, but lose this ability by the CD4+ CD8+ double positive stage. I have focused mechanistic studies on pre-B cells, in which I can readily control the induction of RAG expression and of RAG cleavage at the Igκ AgR locus. These experiments show a rapid decrease in *Rag1* and *Rag2* transcription after pre-B cell exposure to IR. These mRNA changes lead to loss of Rag1 protein, but not Rag2 protein due to the long half-life of Rag2 protein in pre-B cells. However, since RAG endonuclease activity requires both Rag1 and Rag2, the selective loss of Rag1 would be sufficient to suppress RAG cleavage. Accordingly, I have found that DSBs induced by the genotoxic drug etoposide inhibit RAG-induced cleavage at Igκ loci. Rag-induced DSBs in pre-B cells have been previously shown to regulate pre-B cell survival and differentiation by activating the ATM protein and the NF-κB essential modulator (Nemo), promoting NF-κB-dependent gene transcription. I find that suppression of Rag expression in response to exogenous DSBs requires both ATM and Nemo activity, suggesting that this pathway also regulates Rag expression. I propose that this pathway protects developing lymphocytes from potentially oncogenic AgR translocations. I will use my findings to disrupt DSB-induced suppression of V(D)J recombination and determine the effect on AgR translocations and lymphomagenesis.

P15. "Natural killer cells do not always need *STIM*ulation to kill"

Jacquelyn Freund, Ruth Choa, Corbett Berry, Tanner Robertson, Brenal Singh, Janis Burkhardt, Bruce Freedman, Taku Kambayashi

Calcium is a critical second messenger in lymphocytes that contributes to the development, cellular activation, and gene transcription. Stimulation of natural killer (NK) cells through their activating receptors drives the formation of IP_3, which initiates store-operated calcium entry (SOCE). IP3 promotes the oligomerization of stromal interacting molecules 1 and 2 (STIM1 and STIM2) with the calcium-specific ORAI channel, and this is thought to be a principal signaling event for NK cell effector function. In this study, we demonstrate that while SOCE is critical for NK cell interferon gamma (IFNγ) production, SOCE is dispensable for primary NK cell degranulation following activating receptor stimulation or target cell interaction. Murine NK cells stimulated in the absence of extracellular calcium degranulate to activating receptor signals comparably to controls stimulated in 2mM calcium.

Furthermore, treatment of NK cells with phorbol 12-myristate 13-acetate (PMA), which activates DAG-mediated signals, drives CD107a expression on NK cells *in vitro*. Ionomycin, a calcium inonophore, does not induce CD107a expression on NK cells. Using a genetic approach targeting STIM1 and STIM2, NK cells deficient in STIM1/STIM2 (cDKO) have preserved degranulation following ITAM-mediated activating receptor stimulation. cDKO NK cells kill Yac-1 and p815 target cells *in vitro* and clear MHC-I deficient RMA-s cells *in vivo*. As cytotoxic lymphocytes have been reported to require calcium for granule fusion and release events, our observations suggest that calcium release from the endoplasmic reticulum following NK cell receptor activation may be sufficient to trigger NK cell degranulation and killing. Interestingly, the preservation of cytotoxic function is not extended to IL-2 expanded, pre-activated cDKO NK cells. We are currently testing mechanistic hypotheses to determine why SOCE is required for cytotoxicity in IL-2-expanded NK cells, but dispensable for primary NK cells. Together our findings are the first to show that while SOCE is imperative for IFNγ production in NK cells, NK cytotoxic function is preserved in the absence of SOCE.

P16. "Age and Acute Influenza: Clinical characteristics and altered gene expression in pediatric influenza"

Sarah Henrickson, Sasikanth Manne, Douglas V. Dolfi, Kathleen D. Mansfield, Rakesh D. Mistri, Elizabeth R. Alpern, Scott E. Hensley, Kathleen E. Sullivan, Susan E. Coffin, E. John Wherry

Acute respiratory viral infections yield significant morbidity in pediatrics and represent a significant fraction of emergency room admissions. In 2009 we enrolled 29 patients with influenza-like illness from a tertiary care pediatric emergency room and 18 healthy controls. Each ILI patient and had viral testing and all patients had CD8+ T cells sorted from PBMCs for DNA microarray analysis. Of ILI patients, 52% had H1N1 pandemic influenza alone, 21% had H1N1 pandemic influenza and rhinovirus co-infection, 14% had rhinovirus alone and 4 patients had RSV with co-infections (11%; Rhino or Adenovirus) or without (3%) co-infections. Based on clinical history and viral titers, patients did not have protection to H1N1 swine flu prior to this acute illness. Using the DNA microarray data from the influenza-only patients in our study and historical datasets, we developed an influenza pediatric signature (IPS) and compared our signature to many other influenza gene expression signatures. Our signature is sensitive in that is able to detect influenza (including influenza with co-infections) and is effective in both pediatric and adult datasets. It is also specific, in that it is not triggered by other viral infections, both acute (rhinovirus, RSV) and chronic (HIV, HCV). We correlated gene expression with clinical characteristics at presentation (age, weight, gender, need for return to care) and currently (asthma status), with possible mechanistic underpinnings. Finally, while older pediatric patients showed the expected upregulation of interferon signature genes in the setting of acute influenza, we uncovered novel mechanisms underlying disease in young patients with influenza which may reflect primary vs. secondary exposure to flu or additional mechanisms in this age group.

P17. "Successive annual influenza vaccination induces a recurrent oligoclonotypic memory response in circulating T follicular helper cells"

Ramin Sedaghat Herati, Alexander Muselman, Laura Vella, Bertram Bengsch, Kaela Parkhouse, Daniel Del Alcazar, Jonathan Kotzin, Susan A. Doyle, Pablo Tebas, Scott E. Hensley, Laura F. Su, Kenneth E. Schmader, E. John Wherry

T follicular helper CD4 cells are crucial providers of B cell help during adaptive immune responses. A circulating population of CD4 T cells, termed cTfh, have similarity to lymphoid Tfh, can provide B cell help, and responded to influenza vaccination. However, it is unclear whether human vaccination-induced cTfh respond in an antigen-specific manner and whether they form long-lasting memory. Here, we identified a cTfh population that expressed multiple T cell activation markers and could be readily identified by coexpression of ICOS and CD38. Importantly, this subset expressed more Bcl-6, c-Maf, and IL-21 than other blood CD4 subsets. Influenza vaccination induced a strong response in the ICOS+CD38+ cTfh at day 7, and this population included hemagglutinin-specific cells by tetramer staining. Moreover, TCRB sequencing identified a clonal response in ICOS+CD38+ cTfh that correlated strongly with the increased circulating frequency. In subjects who received successive annual vaccinations, a recurrent oligoclonal response appeared in the ICOS+CD38+ cTfh subset at 7 days after every vaccination, unique to the subject and stable over time. These oligoclonal responses in ICOS+CD38+ cTfh after vaccination persisted in the ICOS-CD38- cTfh repertoire in subsequent years, suggesting clonal maintenance in a memory reservoir in the more-stable ICOS-CD38- cTfh subset. These data highlight the antigen-specificity and memory properties of

P18. "Macrophage-Dependent Regeneration of Pulmonary Epithelia Requires Trefoil Factor 2 For Wnt Expression"

Li-Yin Hung, Debasish Sen, Taylor K. Oniskey, Wildaliz Nieves, Anatoly Urisman, Matthew F. Krummel, De'Broski R. Herbert

Coordination between epithelial and myeloid cell lineages may facilitate tissue repair, but mechanistic evidence is lacking. Independently of Type 2 cytokines (interleukin-4/13), and T, B or ILC populations, lung macrophages promoted epithelial proliferation following injury caused by *Nippostrongylus brasiliensis* or bleomycin sulfate. Multiple myeloid populations up-regulated Trefoil factor 2 (TFF2) following lung injury and CD11c-driven *Tff2* deletion impaired the proliferative expansion of pro-SpC^+ distal lung epithelial progenitors. Direct interactions between macrophages and damaged epithelia resulted in non-canonical Wnt production, which accelerated epithelial proliferation, trans-epithelial resistance, and barrier function in a TFF2-dependent manner. In summary, the current study demonstrates that TFF2 is a regenerative cytokine expressed by macrophages to facilitate repair of infectious or non-infectious lung damage.

P19. "Characterizing the origin and the function of tumor-infiltrating macrophages in Ptch1+/- p53-/- medulloblastoma"

Kanika Jain, Mai Dang, Tom Curran, Chi V. Dang, Malay Haldar

Background: Medulloblastoma is one of the most common malignant pediatric brain tumors. It can arise through aberrant activation of sonic hedgehog (Shh) signaling, which has been modeled in *Ptch1+/- p53- /-* mice. A small molecule inhibitor of hedgehog pathway causes significant regression of Shh tumors, but resistance eventually develops. Targeting non-malignant compartments of the tumor microenvironment may provide an alternative therapeutic approach that is less prone to resistance. Recent work in murine glioblastoma models suggests that targeted modulation of the macrophage activation state can antagonize tumor growth. Yet, the contribution of macrophages in medulloblastoma is poorly understood. Hence, we set out to uncover the frequency, distribution, origin, and function of macrophages in medulloblastomas using a *Ptch+/+ p53-/-* mouse model. The overarching goal is to explore whether macrophages can be targeted as a monotherapy or in combination with molecular targeted therapy in medulloblastomas.

Aim: To characterize tumor-infiltrating macrophages in *Ptch1+/- p53-/-* spontaneous murine medulloblastoma, before and after treatment with a sonic hedgehog inhibitor, GDC-0449.

Methods: Sectioned tissues of spontaneous cerebellar tumors from *Ptch1+/- p53-/-* mice were stained for myeloid cell markers F4/80 and Iba1 before and after treatment with various doses of GDC-0449. Flow cytometry analysis was performed on a small number of normal brains, untreated tumors, and GDC-0449 treated tumors to further characterize the infiltrating immune cells.

Result: Immunohistochemistry shows that F4/80 and Iba1 positive cells are present in *Ptch1+/- p53-/-* tumors and their numbers increase significantly after 8 doses of GDC-0449 treatment. $F4/80^+$ cells persist in large numbers in regressed tumors. Notably, tumor-induced disruption in the cytoarchitecture of the cerebellum appears to be largely restored upon tumor regression. Flow cytometry analysis on a small number of untreated and treated tumors shows that these infiltrating cells are mostly macrophages ($CD45^+F4/80^+CD11b^+CD11c^-Ly6G^-Ly6C^-$).

Conclusion: Macrophages are present in growing *Ptch1+/- p53-/-* medulloblastomas. There appears to be a significant increase in their number after treatment with molecular targeted therapy, suggesting they may play a role in drug-induced tumor regression.

Future Directions: Tumor-infiltrating macrophages in medulloblastoma may be derived from resident microglia or from the differentiation of tumor-infiltrating monocytes. Knowing the origin of these macrophages is important because therapeutic strategies modelled to disrupt their development and function would be dependent on their source. Hence, we aim to identify the origin of the infiltrating macrophages by using a combination of immunohistochemistry and flow cytometry techniques. We will also deplete the macrophages in spontaneous tumors before and after treatment with GDC-0449 to uncover their role in tumor growth, in drug-induced tumor regression, and in the restoration of cerebellar cytoarchitecture post-regression.

P20. "Delineating the role of pioneer transcription factors in T cell development"

John L. Johnson, Golnaz Vahedi

The dogma of cell development is that progenitor cells have open, accessible genomes that become condensed and silenced during differentiation in order to commit a cell to a particular lineage. Acting contrary to this dogma is a special class of transcription factors—known as “pioneer transcription factors”—that engage heterochromatic sites in progenitor cells and initiate chromatin changes. These changes include an opening of the chromatin for subsequent binding of other transcription factors to initiate a developmental program. The pioneer transcription factors underlying differentiation in T cells has not been previously characterized. Thus, we set out to investigate the role of pioneer transcription factors in lymphocyte development and to characterize the histone modifications that correspond to their activity. Systematic analysis of genome-wide chromatin accessibility in hematopoietic progenitors and lymphocytes, using ATAC-seq, revealed genomic regions that are initially closed in progenitor cells become accessible upon reaching the CD4+ and CD8+ T cell lineage. The opening of these previously closed genomic areas indicates pioneer transcription factor activity. Analysis of the histone modifications, obtained from ChIP-seq, revealed sequential poising of the chromatin at these regions throughout differentiation. Upon terminal differentiation, these poised modifications are removed from genomic regions corresponding to opposite lymphoid lineages to seal the developmental fate of the cell. Motif analysis of transcription factor binding sites revealed an enrichment of motifs at these genomic regions for certain transcription factors. This will be followed up in further investigation as we continue to uncover the role of transcription factors that act as “pioneers” during T cell development.

P21. "Investigating the Role of Tox in Regulating T Cell Exhaustion"

Omar Khan, E. John Wherry

Chronic infections, such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV), and cancers, including melanoma and non-small cell lung cancer, account for a significant proportion of human mortality. Prolonged exposure to antigen often results in the functional “disarmament” of responding T cells. Termed T cell exhaustion, this alternate differentiation state is characterized by the hierarchical suppression of critical effector functions, dampening of proliferative capacity, and loss of memory differentiation, ultimately limiting the ability of antigen-specific T cells to appropriately combat disease. Though numerous reports have implied important roles for transcription factors (TFs), inhibitory receptors (IRs), and soluble mediators in the development of T cell exhaustion, the molecular mechanisms that regulate the early events of this process remain poorly understood.

Previous work from our group and others has shown that T cells exposed to acute or chronic forms of LCMV have significantly different transcriptional profiles, suggesting a unique differentiation pathway dependent on antigen burden. Our preliminary data suggest that this divergence begins as early as 6 days after exposure to virus and that many of the processes that differ between acute and chronic infection are related to chromatin remodeling. Indeed, several studies have now demonstrated that chronic infection results in global changes in DNA methylation and histone acetylation. These findings suggest that genomic structure may play an important and early role in determining T cell responses to chronic disease. Yet, how and when these structural differences in the T cell genome are established during the course of chronic viral infection remain poorly understood.

Utilizing computational approaches on T cell transcriptional data from mice infected with either acute or chronic LCMV, we identified a highly conserved chromatin-associated protein, Tox, that is strongly upregulated within 6 days of exposure to chronic infection but downregulated in effector and memory T cells. Previous studies have classified Tox as a member of the high-mobility group (HMG) proteins. These proteins, which include Lef-1 and Tcf-1, have been shown to bind and modify local chromatin structure, altering locus transcription. The role of Tox in the immune system remains incompletely characterized and though it has been shown to be critical in regulating the development of NK, innate-like and CD4 T cells, its role in regulating peripheral T cell responses remains unexplored.

Here, we show that, in CD8+ T cells, the expression of Tox is regulated by NFAT and NF- kB-mediated signals.

Moreover, we have found that the downstream targets of Tox include the inhibitory receptor PD-1 and the transcription factor Eomes. Over-expression of Tox increases the expression of both of these proteins. As PD-1 and Eomes play a critical role in orchestrating the dysfunction associated with exhaustion, our preliminary results suggest that Tox may be a key upstream regulator of this process.

P22. "Quantitative proteomics predicts functional consequences of ubiquitylation events during T cell stimulation"

Jonathan Kotzin, Sean Spencer, Sam McCright, Walter Mowel, Arjun Raj, E. John Wherry, Amy Klion, Adam Williams, Richard Flavell, Jorge Henao-Mejia

Neutrophils, eosinophils and 'classical' monocytes collectively account for ~70% of human blood leukocytes and are among the shortest-lived cells in the body. Precise regulation of the lifespan of these myeloid cells is critical to maintain protective immune responses and minimize the deleterious consequences of prolonged inflammation. However, how the lifespan of these cells is strictly controlled remains largely unknown. Here we identify a long non-coding RNA that we termed *Morrbid*, which tightly controls the survival of neutrophils, eosinophils and classical monocytes in response to pro- survival cytokines in mice. To control the lifespan of these cells, *Morrbid* regulates the transcription of the neighbouring pro-apoptotic gene, *Bcl2l11* (also known as *Bim*), by promoting the enrichment of the PRC2 complex at the *Bcl2l11* promoter to maintain this gene in a poised state. Notably, *Morrbid* regulates this process in *cis*, enabling allele-specific control of *Bcl2l11* transcription. Thus, in these highly inflammatory cells, changes in Morrbid levels provide a locus-specific regulatory mechanism that allows for rapid control of apoptosis in response to extracellular pro-survival signals. As *MORRBID* is present in humans and dysregulated in individuals with hypereosinophilic syndrome, this long non-coding RNA may represent a potential therapeutic target for inflammatory disorders characterized by aberrant short-lived myeloid cell lifespan.

P23. "The Role of Trib1 in Granulocyte Development"

Ethan A. Mack, Sarah J. Stein, Kelly S. Rome, Warren S. Pear

The development of granulocytes, including eosinophils and neutrophils proceeds with the concerted action of key transcription factors including C/EBP family members, GATA factors, and PU.1, yet how their expression is regulated is unclear. We and others identified tribbles homologue 1 (Trib1) as a critical regulator of C/EBPα protein turnover and others have shown that whole body Trib1-deficient mice lack eosinophils and expand neutrophils, but the developmental stage and mechanism of regulation remain unexplored. We generated mice lacking *Trib1* in the hematopoietic compartment using Vav-Cre ($VCT1^{fl/fl}$). Using these mice, we employed both *in vivo* and *ex vivo* approaches to assess eosinophil development and regulation. We confirmed that $VCT1^{fl/fl}$ mice have a near complete absence of eosinophils in the blood, spleen, and peripheral sites. This correlates with an expansion of neutrophils. In addition, we found that the efficiency of generating eosinophils *ex vivo* in culture with IL-5 is dramatically reduced compared to wild-type bone marrow. In contrast, Trib1-deficient bone marrow readily generated neutrophils in IL-5 culture. This correlated with increased C/EBPα p42 protein in Trib1-deficient eosinophils and neutrophils. During murine eosinophil differentiation from myeloid progenitors, Trib1 expression increased at the eosinophil progenitor (EoP) stage, suggesting that Trib1 regulates eosinophil differentiation after the GMP stage. These data raise the possibility that graded C/EBPα expression is required to balance eosinophil and neutrophil differentiation. Together these data suggest that Trib1 may play a key role in regulating granulocyte identity.

P24. "The ubiquitin ligase Itch negatively regulates GC B cell persistence"

Emily Moser, Michael Cancro, and Paula Oliver

The NIH estimates that greater than 2.3 million Americans suffer from autoimmune diseases, which arise due to a wide variety of etiologies, often caused by combinations of multiple genetic inputs and environmental factors. However, in some cases, a single gene mutation is sufficient to drive autoimmunity. In both humans and mice, deletion of the E3 ubiquitin ligase Itch is sufficient to drive high levels of serum antibody and autoimmunity. Although Itch is known to be a potent regulator of antibody responses, thus far, no studies have evaluated

whether or how Itch regulates B cell fate or function *in vivo*. We now show that Itch limits the magnitude of the germinal center (GC) B cell population. We examined splenic B cell populations in Itch- deficient and control mice and determined that antigen-experienced B cells (i.e. GC and memory B cells) were elevated in number, whereas the frequencies of other B cell populations (i.e. transitional, marginal zone, and follicular B cells) were comparable to wild type controls. Mixed BM chimeras revealed that Itch played a negative regulatory role within B cells to limit GC B cell numbers. To track the generation and persistence of GC B cells, we transferred transgenic NP-specific B cells that lacked Itch, or control cells, into mice and immunized with NP-ova. We found that while antigen specific B cells expand equally, regardless of their ability to express Itch, Itch KO GC B cells accumulated late in the response, persisting after the WT NP-specific cells had waned. Furthermore, these cells exhibited evidence of altered BCR selection. Current studies are aimed at defining the molecular mechanisms by which Itch regulates GC B cell persistence and the role of Itch in altered selection and production of autoantibodies. These studies have implications for autoimmune disease and vaccine development.

P25. "A novel lncRNA promotes group 1 innate lymphoid cell maturation infections"

Walter Mowel, Jorge Henao-Mejia

Innate lymphoid cells (ILCs) are located throughout the body and are critical for defense against a variety of pathogens. Group 1 ILCs, including natural killer (NK) cells and ILC1s, are particularly important for defense against intracellular infections and cancer immunosurveillance. However, the factors regulating the development, maturation, and function of these cells are not well understood. Long noncoding RNAs (lncRNAs) are expressed in a cell type-specific manner and are key regulators of gene expression programs. In this work, we identify a genomic region encoding a lncRNA that is specifically expressed in group 1 ILCs, but not ILC2s or ILC3s. We show here that group 1 ILCs from mice deficient in this region were dramatically reduced in number, were functionally deficient in vitro and in vivo, and lack peripheral NK cells due to a block in their maturation. Furthermore, this cell-intrinsic phenotype was associated with altered chromatin accessibility and gene expression. Finally, this was specific to group 1 ILCs as both the homeostasis and function of ILC2 and ILC3 populations was unaltered in lncRNA-deficient mice. Collectively, our data reveals a critical role for a novel lncRNA-encoding regulatory element in the homeostasis of group 1 ILCs.

P26. "Synergy of LAG3 and PD1 in T cell exhaustion: mechanisms and applications"

Shin Foong Ngiow, Kristen E. Pauken, Sasi K. Manne, E. John Wherry

T cell exhaustion is a key feature of chronic infection and cancer, where anti-viral/cancer T cells progressively turned dysfunctional. Conversely, T cell exhaustion could be reversed by the blockade of inhibitory receptors like PD1 and/or LAG3. Notably, these anti-viral/tumor T cell responses are dramatically enhanced when PD1 and LAG3 are targeted simultaneously. This therapy approach of targeting multiple inhibitory receptors has gained great successes in the clinic, with patients demonstrating improved survival benefits. However, little is known about the cellular and molecular mechanisms by which PD1 and LAG3 regulate T cell exhaustion, let alone the understanding of synergy between these receptors. For this project, we will use the well-established lymphocytic choriomeningitis virus (LCMV) model of chronic infection, and a series of gene-targeted LCMV-specific T cells to assess the synergy between PD1 and LAG3 in directing T cell exhaustion. By exploiting LCMV-specific T cells in this model of chronic infection, we can accurately define the T cell intrinsic and extrinsic mechanisms elicited by PD1 and LAG3 inhibitory pathways. In addition, we can define if targeting PD1 and LAG3 simultaneously amplifies the cellular and molecular response of “reversible” exhausted T cells or whether dual blockade reinvigorates cells otherwise non-reversible by either blockade alone. The experiments proposed herein will provide critical insight into how T cell potency and longevity is enhanced when PD1 and LAG3 are co-targeted, notably in the context of immune checkpoint therapy in cancer.

P27. "Modeling anti-CD20 chimeric antigen receptor therapy in canine B cell lymphoma patients"

M. Kazim Panjwani, Josephine Gnanandarajah, Martha A. MaloneyHuss, Nicola J. Mason

Chimeric antigen receptor (CAR) therapy has demonstrated great promise in treating human leukemias, but preclinical murine models are limited in their ability to predict safety and efficacy in humans. Given the rapid and on-going advances in CAR T technology in the laboratory, it now becomes necessary to identify and develop an outbred, large animal spontaneous cancer model in which the safety of novel targets and therapeutic effectiveness of re-directed T cells can be evaluated and optimized.

Canines naturally develop spontaneous B cell lymphoma, and we have previously shown the feasibility of evaluating CAR T cell therapy in dogs using CD20-targeting RNA CAR T cells. To parallel human CAR T cell approaches, we have now optimized primary canine T cell transduction with lentivirus. Once peak T cell activation and artificial antigen presenting cell elimination timeframes were established, transduction yielded up to 26% CAR+ canine T cells. Canine T cells transduced with a second generation CD20-targeting CAR (CD20-28-ζ) demonstrated CAR-mediated, antigen-specific proliferation and efficient cytolysis of a CD20+ canine B cell lymphoma line *in vitro*.

Three relapsed canine B cell lymphoma patients were treated with autologous CD20-28-ζ CAR T cells. Despite the administration of low numbers of CAR T cells, transient anti-tumor effects were observed in the first 2 patients. Following administration of ~700,000 CAR T cells/kg intravenously to the third patient, CAR T cells were found to persist and expand in the periphery and malignant nodes and disease progression was halted. This work establishes the feasibility of using the dogs with spontaneous cancer as pre-clinical models for advancing human CAR T cell therapy.

P28. "Detection of bacterial ligands by human inflammasome"

Valeria M. Reyes-Ruiz, Sunny Shin

The innate immune response is critical for antibacterial defense and can be initiated by the engagement of pattern recognition receptors by pathogen-associated molecular patterns. 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 (hNAIP). Previous studies found that the T3SS needle protein was the only bacterial ligand that activates the NAIP inflammasome in humans. Surprisingly, our preliminary studies suggest that the T3SS inner rod (PrgJ) and flagellin also activate the hNAIP inflammasome. Future experiments will define how a single human *NAIP* gene can allow for recognition of the three different bacterial ligands. Additionally, we will define inflammasome responses to PrgJ homologs from other bacterial species. The results of these studies will offer a better understanding of bacterial sensing by the inflammasome in human cells.

P29. "ERM-deficient T cells display enhanced memory formation but poor activation following stimulation through the T cell receptor"

Tanner Robertson, Janis K. Burkhardt

Ezrin, radixin, and moesin (ERM) proteins create specialized membrane subdomains by linking phosphatidyl inositol lipids and protein binding partners in the plasma membrane to the underlying actin cortex. These proteins are widely expressed and have well-characterized roles in cell polarity, development, and cytokinesis. In recent years, it has become clear that these proteins are also intimately involved in signal transduction. In humans, mutations in ERM proteins cause severe immunodeficiency characterized by lymphopenia and an abnormally high ratio of memory to naïve T cells. To investigate the role of ERM proteins in T cell activation, we generated mice

with T cells deficient in ezrin and moesin, the two ERM family members expressed by T cells. Preliminary analysis shows that the memory population in these mice is significantly enlarged. Curiously, however, T cells from these mice activate poorly in response to stimulation through the T cell receptor. Further characterization of these T cells will be described, along with biochemical analysis aimed at understanding the molecular mechanisms through which ERM proteins control T cell responses.

P30. "Differential Homing Dynamics of Short and Long Lived Plasma Cells in the Bone Marrow and Elsewhere"

Rebecca Rosenthal, David Allman

Long term survival of plasma cells is thought to require a specific bone marrow survival niche. It is unclear if plasma cells remain in this niche their entire lifespan, or if they can recirculate through the body before returning. As large numbers of plasma cells are often mobilized after infection or vaccination, determining their recirculation capacity is important. To investigate this, we performed parabiosis (joining) surgery on mice with disparate CD45 alleles. Joined mice establish a shared circulatory system and we find partner derived short and long lived plasma cells in their bone marrow, with partner derived long lived plasma cells at lower frequencies than newly derived short lived plasma cells. Surprisingly, we found a similar bias in partner derived long versus short lived plasma cells in the spleen, implying the spleen may serve as a long lived plasma cell survival niche. These data imply long lived plasma cells can recirculate through the bloodstream before returning to survival niches in the bone marrow or possibly the spleen. However, the possibility we are detecting newly formed rather than recirculating plasma cells remains an important caveat.

P31. "Crk Adapter Proteins Orchestrate LFA-1 Outside-in Signaling and Mechanosensing in T cells"

Nathan H. Roy, Joanna L. MacKay, Daniel A. Hammer, and Janis K. Burkhardt

T cells must respond appropriately to many different cues on the vascular endothelium during the process of transmigration. These include small molecules and membrane receptors present on the endothelial surface, as well as the biophysical properties of the endothelial monolayer itself. The integrin LFA-1 on T cells is known to mediate firm adhesion and transmigration, but although the mechanisms of integrin activation are well studied, the downstream signals from bound integrins leading to cell spreading, crawling, and ultimately transmigration, are largely unknown. We have previously reported that Crk family adapter proteins are important regulators of T cell transmigration, but the mechanism remained elusive. Here, we identify Crk proteins as key mediators of integrin outside-in signaling. Primary mouse $CD4^+$ T cells that lack Crk proteins fail to spread on ICAM-1 coated surfaces and have defects in LFA-1 dependent cell migration. By analyzing downstream signaling pathways, we have found that PI3K signaling is severely diminished in the absence of Crk proteins, while ERK signaling remains intact. This results in an abnormal distribution of PIP_3 in migrating cells, as well altered Rho family GTPase activity, likely accounting for the spreading and migration defects. Additionally, we found that Crk proteins promote the phosphorylation and activation of CasL, a homolog of a known force sensing protein, suggesting that Crk proteins may mediate mechanotransduction of LFA-1 signals. Using ICAM-1 coated hydrogels of varying stiffness, we found that WT $CD4^+$ T cells display a mechano- sensitive response to ICAM-1, while Crk deficient T cells do not. Our data indicate a major role for Crk proteins in transducing integrin outside-in signals, and reveal a role for Crk proteins in T cell mechanosensing. These data provide new insights into the molecular mechanisms used by T cells to scan the endothelial wall for permissive sites for transmigration.

P32. "The loss of DGKζ impairs ILC2 function"

Brenal Singh, Taku Kambayashi

The generation of diacylglycerol (DAG) is critical for promoting immune cell activation, regulation, and function. Diacylglycerol kinase ζ (DGKζ) serves as an important negative regulator of DAG by enzymatically converting DAG into phosphatidic acid (PA) to shut down DAG-mediated signaling. However, while the enhancement of DAG signaling is thought to augment immune cell function, the loss of DGKζ can result in both immunoactivation and

immunomodulation depending on the cell type and function. Group 2 innate lymphoid cells (ILC2) are critical mediators of type 2 immune responses, such as those present in allergy and asthma. Here, we demonstrate that the loss of DGKζ suppresses ILC2 function. DGKζ KO ILC2s have impaired cytokine production in response to IL-33 stimulation. Furthermore, the loss of DGKζ attenuates papain-induced airway inflammation, as measured by reduced eosinophil and ILC2 accumulation in the lungs and decreased Th2 cytokine levels in the bronchoalveolar lavage (BAL) fluid of protease-challenged mice. Our data demonstrates that DGKζ serves as a novel regulator of ILC2 function.

P33. "microRNA-155 regulates CD8 T cell responses to chronic viral infections"

Erietta Stelekati, Zeyu Chen, Sasikanth Manne, Makoto Kurachi, Mohammed-Alkhatim Ali, Keith Lewy, Jennifer L. Hope, Adam Fike, Donald T. Gracias, Peter D. Katsikis, E. John Wherry

Persistent pathogenic viral infections are a major cause of morbidity and mortality worldwide. CD8 T cells are crucial mediators of antiviral immunity. However, during persistent infections, chronic antigen stimulation results in dysfunctional, “exhausted” T cells that cannot provide protection. Here, we identified microRNA(miR)-155 as a key regulator of CD8 T cell responses during chronic LCMV infection. Overexpression of miR-155 enhanced and prolonged the expansion of LCMV-specific CD8 T cells, resulting in a large and long-term persisting population of LCMV-specific CD8 T cells, even in the presence of high viral loads. Interestingly, overexpression of miR-155 increased the expression of inhibitory receptors and the transcription factor Eomes, consistent with a more prominent exhaustion phenotype. Transcriptional profiling revealed key cellular signalling pathways downregulated by miR-155, including inflammatory pathways and pathways downstream of TCR signaling. Therefore, we suggest that miR-155 plays a crucial role in regulating CD8 T cell responses to antigenic and inflammatory signals in order to sustain the pool of exhausted CD8 T cells long-term during chronic infection.

P34. "Maintenance of X chromosome inactivation in female B lymphocytes"

Camille M Syrett, Vishal Sindhava, Arindam Basu, Michael Atchison, Michael P. Cancro, Montserrat C. Anguera

X-chromosome inactivation (XCI) equalizes the dosage of X-linked genes between XX females and XY males. During XCI a single female X chromosome is transcriptionally silenced, generating an epigenetically distinct inactive X (Xi). In somatic cells, XCI is maintained by continued expression of Xist RNA and by the deposition of heterochromatin marks on the Xi. We recently made the remarkable discovery that female B lymphocytes maintain XCI differently than all other adult somatic cells. Surprisingly, naïve B cells have unusual patterns of Xist RNA localization and lack Xi heterochromatin. To determine the mechanisms underlying this abnormal XCI maintenance, we analyzed Xist RNA localization of progenitor populations in the developing B cell lineage. We observed that canonical Xist RNA localization is disrupted at the pro-B cell stage through the mature naïve stage. In these cells, Xist RNA transcripts and heterochromatin marks begin to cluster on the Xi after 16 hours of stimulation. Furthermore, we found that the transcription factor Yy1 is necessary to recruit Xist RNA to the Xi. Together, these data indicate that the maintenance of XCI is a dynamic process in female lymphocytes. Future experiments will determine how this novel maintenance affects expression of X-linked genes on the Xi.

P35. "Antiviral roles for the DEAD-box helicases DDX24 and DDX56 in RNA virus infection"

Frances Taschuk, Ryan Moy, Ari Yasunaga, and Sara Cherry

Innate immune sensing relies on interaction of pathogens with host proteins. DEAD-box helicases are nucleic acid binding proteins which include viral RNA sensors RIGI, MDA5, and DDX17. RNAi screening of DEAD-box helicases identified DDX24 and DDX56 as antiviral factors. I have shown that DDX24 and DDX56 are antiviral against Chikungunya virus (CHIKV), Dengue virus, and Zika virus, but are not required for activation of interferon or induction of interferon- stimulated genes. DDX24 and DDX56 are nucleolar proteins; to further explore their cell biology, I investigated their localization during viral infection. I found that CHIKV infection disrupts the nucleolus, dispersing these and other nucleolar proteins throughout the nucleus. We tested whether a specific CHIKV protein, nsP2, which localizes to the nucleus, was sufficient to disrupt nucleoli and found that ectopic nsP2 expression phenocopied infection. Future work will determine the functions of CHIKV nsP2 required for nucleolar

disruption and the effect on rRNA biogenesis. I will also address the antiviral mechanism of DDX24 and DDX56 by examining whether they bind viral RNA or have an indirect effect.

P36. "Regulation of proliferation by TCF-1 and p21 in thymocytes after Beta-selection"

Brittany Townley, Avinash Bhandoola

T-cell factor 1 (TCF-1, TCF7) is highly upregulated in early thymic progenitors and remains stably expressed in the double negative (DN) to double positive (DP) T cell populations. Although TCF-1 has been shown to confer T-cell specification in progenitor populations, the role of TCF-1 in later T cell development is not well understood. In a CD2-Cre $TCF7^{f/f}$ mouse model, conditional deletion of TCF-1 prior to beta selection results in reduced thymic cellularity, with an apparent failure at the beta selection checkpoint. Further characterization reveals that this defect is due to reduced proliferation post beta selection. Interestingly, P21 RNA expression increases relative to control, concurrent with an increased percentage of cells in G0. Because p21 functions as a cell cycle inhibitor at the G1 checkpoint, we used a CD2-Cre $TCF7^{f/f}$ $p21^{-/-}$ mouse model to determine whether loss of p21 rescues the phenotype resulting from loss of TCF-1 at beta selection. Our results thus far indicate that the double knockout shares a similar phenotype to the TCF-1 conditional knockout, as T-cell development in the double knockout is not rescued. Future work will focus on determining why p21 is upregulated in response to loss of TCF-1, and whether loss of p21 abrogates the proliferative block resulting from loss of TCF-1. Collectively, this work will provide further insight to the role of TCF-1 and cell cycle regulation at beta selection.

P37. "Commensal Microbiota Tune Systemic Toll-Like Receptor-Mediated Inflammatory Responses"

Lehn K. Weaver, Chhanda Biswas, Edward M. Behrens

Background/Purpose: Although commensal microbiota are thought to contribute to the development of autoimmunity, the cellular and molecular mechanisms connecting changes in gut microbiota to the development of autoreactive adaptive immune responses remain elusive. Preclinical models of autoimmunity demonstrate reduced disease severity and defective adaptive immune responses in autoimmune-prone mice treated with antibiotics or housed in germ-free conditions. As many autoimmune diseases show evidence of chronic Toll-like receptor (TLR) activation, we posited that defects in TLR-driven innate immune responses may explain the defective adaptive immune responses and reduced disease severity seen in antibiotic-treated mice.

Methods: We tested our hypothesis by using two murine models of TLR-driven systemic inflammation in mice treated with or without broad-spectrum antibiotics. Analysis of systemic inflammation was performed 10-14 days after a single dose of pristane (TLR7-driven inflammation) or after 5 doses of CpG (TLR9-driven inflammation) in mice treated with or without antibiotics. Systemic inflammation was determined by analysis of diffuse alveolar hemorrhage, cytopenias, hypercytokinemia, hepatosplenomegaly, and inflammation-induced myelopoiesis, which develop in these models of TLR-driven inflammation. TLR9 responsive cells isolated from mice treated with 0-5 doses of CpG were analyzed for production of IL-12 following *ex vivo* stimulation with CpG.

Results: Mice treated with broad-spectrum antibiotics were protected from developing systemic immunopathology following TLR-driven inflammation *in vivo*, as evidenced by lack of diffuse alveolar hemorrhage, cytopenias, hypercytokinemia, and hepatosplenomegaly following injection of pristane or repeated doses of CpG. This was not from baseline defects in innate immune cell numbers or TLR responsiveness, as numbers of TLR responsive cells and TLR-driven cytokine production following a single dose of CpG were preserved in antibiotic-treated mice. However, antibiotics abolished the inflammation-induced myelopoiesis and expansion of peripheral TLR responsive monocytes that accompanies both pristane- and CpG- induced immunopathology.

Conclusion: We demonstrate that antibiotic-treated mice have normal baseline responses to TLR-driven inflammatory signals, but fail to develop end-organ damage or sustained systemic inflammation to chronic TLR triggers *in vivo*. Disease protection in antibiotic-treated mice correlated with defective inflammation-induced myelopoiesis and inhibition of peripheral monocyte expansion that drive ongoing TLR immune responses in these

contribute to the development of autoimmunity by tuning systemic innate immune responses in the setting of chronic TLR stimulation.

P38. "Using GATA1-dependent erythropoiesis to determine unique and overlapping functions of the chromatin reading BET family"

Michael T. Werner, Sarah C. Hsu, Aaron J. Stonestrom, Gerd A. Blobel

BET proteins (BRD2, BRD3, and BRD4) bind active, acetylated chromatin and have emerged as potent epigenetic drug targets in various cancers and inflammatory diseases. By modulating the chromatin environment, therapeutic BET protein inhibitors can succeed in what was largely considered impossible – impacting the function of disease-associated transcription factors. The current BET inhibitors target all three family members equivalently by competitively displacing their acetyl-binding bromodomains from chromatin. Despite the utility of these drugs, little is known mechanistically about how BET proteins function or whether they have individual roles in gene transcription. Furthermore, BET inhibition is not completely benign, with early clinical studies reporting anemia and thrombocytopenia as the chief toxicities. Given that GATA1 is the master transcription factor specifying terminal erythroid (and megakaryocyte) differentiation, we are pursuing functional and mechanistic studies to determine which BET proteins are critical to GATA1 function and which protein domains convey isoform specificity. A prior study indicated that BET proteins have an overall role in facilitating GATA1 chromatin binding genome-wide. To determine the role of individual BET proteins, we used CRISPR-Cas9 to generate BRD2- and BRD3-knockouts in a GATA1-inducible erythroid precursor cell line that mimics physiological terminal erythroid differentiation. We were unable to generate a BRD4-knockout cell line but were able to achieve strong BRD4-knockdown using shRNA-mediated depletion. Using these reagents, we performed a series of gene-rescue studies to determine which BET proteins can functionally compensate for each other. By rescuing with either truncated or chimeric BET proteins, we further mapped the individual protein domains that convey these functional differences. These studies indicate that BRD2 and BRD3 are functionally similar to each other but distinct from BRD4. Moreover, their distinct function is bromodomain- independent and can be mapped instead to protein-protein interaction domains. We are currently performing protein interaction and chromatin immunoprecipitation studies to determine how these domains target BET proteins to chromatin and influence transcription. Our gain-of-function cellular system provides a sensitive, physiological, and clinically relevant assay for studying BET proteins. It is likely that the rules governing the interaction between GATA1 and BET proteins in this system will hold true for other transcription factors whose binding profiles strongly correlate with BET proteins, as demonstrated in myeloid cells. We hope that dissecting the mechanism of individual BET proteins will instruct the development of inhibitory compounds that can avoid or selectively exploit perturbations in gene transcription in a predictable manner.

P39. "Recombination signal sequences shape antigen receptor repertoires and enforce allelic exclusion"

Glendon Wu, Katherine Yang-Iott, Craig Bassing

B and T lymphocytes are constituents of the vertebrate adaptive immune system and are defined by their expression of unique antigen receptors (AgRs) on the cell surface. AgR loci are arrays of variable (V), diversity (D), and joining (J) gene segments that the RAG endonuclease assembles into complete AgR genes in the process of V(D)J recombination. RAG binds to and cleaves at recombination signal sequences (RSSs) that flank gene segments. V(D)J recombination of *Tcrb*, *Igh*, and *Igk* loci is regulated such that functional AgR genes are assembled on and expressed from one allele (allelic exclusion) in most cells. It is assumed that allelic exclusion is achieved by mono-allelic initiation and feedback inhibition of the V-to-(D)J recombination step, and intrinsic features of RSSs have been proposed to mediate mono-allelic V-to-DJ recombination of *Tcrb* and *Igh* loci. To investigate the role of RSSs in enforcing allelic exclusion, we established and analyzed mice with the *Trbv2* or *Trbv31* RSS replaced by an RSS that possesses greater intrinsic recombination activity. Each of these RSS substitutions causes a profound increase in the development of T cells expressing either Trbv2$^+$ or Trbv31$^+$ TCRβ chains at the expense of cells expressing other TCRβ chains, reflecting that the RSSs flanking *Trbv* segments are major determinants of *Trbv* recombination frequency. Each RSS replacement also promotes a modest increase in the fraction of cells

expressing Trbv2$^+$ or Trbv31$^+$ TCRβ chains along with another type of TCRβ chain (allelic inclusion). Strikingly, mice with *Trbv2* and *Trbv31* RSS replacements on opposite alleles have a 30-fold higher than normal level of allelic inclusion for Trbv2$^+$ and Trbv31$^+$ TCRβ chains, demonstrating that the RSSs flanking *Trbv* segments enforce TCRβ allelic exclusion. We conclude that the infrequency of *Trbv* recombination as determined by inherent inefficiencies of *Trbv* RSSs limits bi-allelic assembly of *Tcrb* genes within the time window before feedback inhibition permanently cements allelic exclusion.

P40. "Fas expression in memory CD8+ T cell subsets augments cellular differentiation and effector function"

Tori Yamamoto, Nicholas Restifo

Fas is expressed by all memory CD8$^+$ T cell (T_{Mem}) subsets despite their phenotypic and functional heterogeneity. Based on our previous work investigating the non-apoptotic functions of Fas in naïve CD8$^+$ T cells, we sought to elucidate the roles of Fas signaling in T_{Mem} subsets. Augmenting Fas signaling in T stem cell memory (T_{SCM}) and T central memory (T_{CM}) using an oligomerized form of Fas ligand (FasL) enhanced cellular differentiation, promoted the loss of IL-2 production and induced apoptosis. Conversely, antibody blockade (anti-FasL) of Fas signaling in T_{CM} and T effector memory (T_{EM}) produced phenotypically and functionally less differentiated cells and lead to greater fold expansion. Using transgenic mice expressing a mutant Fas lacking a transmembrane cysteine residue (FasC194V) that is unable to undergo S- palmitoylation and aggregate in lipid rafts on a Fas-deficient *lpr* background, we found FasC194V T_{Mem} still undergo cellular differentiation in the absence of death signaling. Microarray analysis demonstrated that T_{Mem} expanded with anti-FasL expressed greater levels of memory-associated transcription factors relative to IgG-treated controls and significantly altered the metabolic state of activated T_{Mem}, most notably by limiting the acquisition of a glycolytic metabolism. Using the Pmel-1 CD8$^+$ TCR transgenic model, we found that Pmel-1 T_{Mem} cultured *ex vivo* with anti-FasL expanded to higher levels *in vivo,* acquired a less exhausted phenotype, and more effectively treated established B16 melanoma tumors compared to controls. Similarly, T_{Mem} expanded with anti-FasL and genetically engineered with an anti-CD19 chimeric antigen receptor exhibited reduced differentiation and augmented anti-lymphoma and anti-leukemia activity compared to controls. These studies demonstrate that Fas signaling promotes not only cell death but also T_{Mem} differentiation, a finding that has implications for the design and execution of T-cell-based immunotherapies in patients with cancer or infectious disease.

P41. "Differential regulation of CD8 T cell priming and effector function during Leishmania infection"

Scarlett Y. Yang, Erika J. Crosby, Fernanda O. Novais, Phillip Scott

Leishmaniasis is a protozoal disease that affects millions of individuals worldwide. Although immune responses are required to control the parasites, excessive immune activation can lead to severe pathology. In a series of studies with experimental murine models and leishmaniasis patients, our lab has shown that CD8 T cells can be either protective or pathologic. Thus, while IFN-γ producing CD8 T cells enhance protection, cytolytic, granzyme B (GzmB) producing CD8 T cells promote increased pathology. Furthermore, by co-infecting mice with lymphocytic choriomeningitis virus (LCMV) we found that bystander LCMV-specific cytolytic CD8 T cells also promote increased leishmanial lesion pathology, while *Listeria monocytogenes* co-infection did not induce pathologic CD8 T cells. These results have prompted us to investigate the factors that promote differentiation of CD8 T cells into either a protective (IFN-γ producing) or pathologic (cytolytic) phenotype. In preliminary studies, we found that IL-15 enhanced GzmB production by CD8 T cells, while IL-12 and IL-18 enhanced IFN-γ production. Our current studies are testing the influence of both cytokines and the pathogen on the differentiation of naïve CD8 T cell into effector cells. To accomplish this, CD8 OT1 cells are cultured with dendritic cells infected with either *Leishmania major* or *Listeria monocytogenes* that express ovalbumin, and the proliferation and phenotype of the CD8 OT1 cells is assessed. These studies will define components controlling CD8 T cell differentiation and effector function in the context of distinct type 1 immune responses.

P42. "Using Proteomics to Identify Novel Host Antiviral Proteins"

G. Renuka Kumar, Jeff Johnson, Melanie Ott, Nevan J. Krogan, Sara Cherry, Holly R. Ramage

Identification of the host proteins and complexes that physically associate with viral proteins will greatly improve our understanding of antiviral mechanisms. We identified HCV-host protein- protein interactions using an affinity-tag purification and mass spectrometry approach. Our computational analysis yielded ~150 high-confidence target host proteins, with interaction partners identified for each of the HCV proteins. This screen was coupled to a lentivirus- mediated shRNA knock-down platform in HCV-infected Huh7.5 hepatoma cells to determine which of these target proteins are most critical to infection. Importantly, our results identify previously known, as well as newly identified interacting host proteins, as novel regulators of HCV infection. We identified several host proteins that result in increased HCV infection upon knockdown, indicating that they play significant roles in the HCV life cycle by restricting viral infection. In an effort to identify host factors engaged by multiple viruses, we are conducting similar studies to find host proteins interacting with other Flaviviridae family viruses, including Dengue and West Nile virus. Results from these screens will allow us to identify novel antiviral host pathways.

P43. "CYTOF2"

Takuya Ohtani

The CyTOF2 is the newest generation mass cytometer available to the Penn and VA community. The technology uses Time-Of-Flight mass spectrometry of rare isotopes as a readout. Practically, in contrast to flow cytometry, the use of metal isotope-labeled antibodies instead of fluorescent–labeled antibodies, allows the separation of a large number of isotope-labelled reagents (currently, more than 40 different isotope labels are available). This technology enables multi-parametric high-dimensional single cell analysis of >40 markers per cell, with minimal background and compensation issues.

The CyTOF Core offers a wide variety of CyTOF-related services including consultation, training, antibody-conjugation, and data acquisition. Please contact Takuya Ohtani, takuya@mail.med.upenn.edu, for details.

P44. “The Human Immunology Core”

Ling Zhao

The Human Immunology Core facility offers a wide range of services and primary human cell products. We provide infrastructure support for early-phase clinical trials. Our well trained and experienced lab staff perform sophisticated immunological assays, such as multiplex Luminex assay, flow cytometry, TCR sequencing, etc. We not only assist with generation of pilot data and data analysis for grant applications, but also assist investigators with the experimental design, and development, validation and implementation of standard operating procedures for clinical trial sample processing, management, shipping and immunology assays. We are constantly implementing newest technologies and provide scientific and technical consultation to investigators.

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