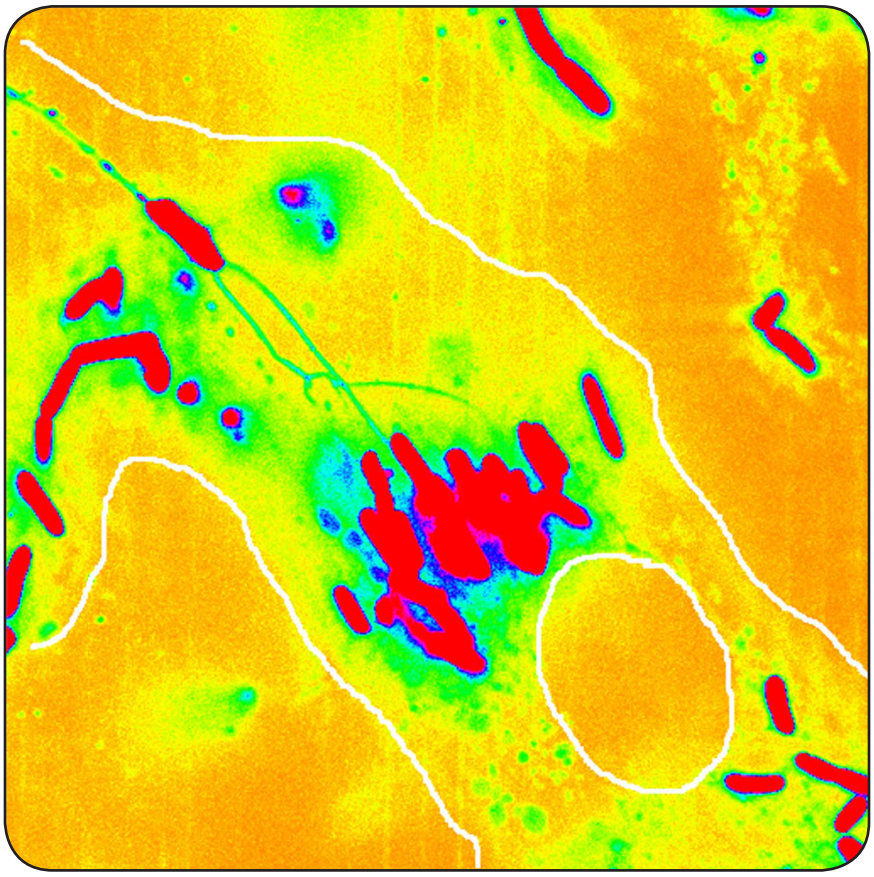
IMMUNOLOGY GRADUATE GROUP

28TH ANNUAL RETREAT

OCTOBER 23 - 25, 2015 | ST. MICHAELS, MD



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FRIDAY, OCTOBER 23

11:00 AM - 1:30 PM	Registration Open
12:00 – 1:15 PM	Lunch
1:20 – 1:30 PM	Welcome Paula Oliver, Ph.D. and Andrew Wells, Ph.D. Retreat Co-Chairs
1:30 – 3:10 PM	Session I: Immune System Development and Function S1A. Caroline Bartman (session chair) "Enhancer regulation of transcriptional bursting parameters revealed by forced chromatin looping" S1B. Megan Fisher "DNA Double-Strand Breaks Suppress Expression and Activity of the RAG Recombinase in Pre-B Cells" S1C. Jacquelyn Freund "Activating receptor signaling induces MHC I-binding inhibitory receptor expression by natural killer cells" S1D. Ethan Mack "The Role of Trib1 in Granulocyte Development" S1E. Walter Mowel "A novel lincRNA is a key regulator of group 1 innate lymphoid cell maturation"
3:30 – 5:10 PM	Session II: Host-Pathogen Interactions S2A. Awo A.K. Layman (session chair) "When Ndfip1 is away, pathogenic Th17s will play: exploring the role of the ubiquitin cascade protein, Ndfip1, in Th17 abundance and pathogenicity" S2B. Thomas Fung "Defining interactions between lymphoid tissue-resident commensal bacteria and the immune system" S2C. James Knox "HIV infection induces the expansion of T-bet expressing B lymphocytes"

	Session II: Host-Pathogen Interactions
3:30 – 5:10 PM	S2D. Lance Peterson "The Activation of Programmed Cell Death During Yersinia Infection Promotes Antibacterial Immunity" S2E. Yan Sun, Ph.D. "Immunostimulatory Defective Viral Genomes from Respiratory Syncytial Virus Promote a Strong Innate Antiviral Response During Infection in Mice and Humans"
5:30 – 6:45 PM	Dinner
7:00 – 9:00 PM	Faculty Talks Jorge Alvarez, Ph.D. "The role of the blood meningeal barrier in supporting neuroinflammatory responses" Erica Stone, Ph.D. "The role of FOXO1 in T cell differentiation and function" Golnaz Vahedi, Ph.D. "Pioneer Transcription Factors in T Cells"
9:00 PM – 12:00 AM	Reception

SATURDAY, OCTOBER 24

8:00 AM - 9:00 AM	Breakfast
9:00 – 10:40 AM	Session III: Translational Immunology S3A. Kazim Panjwani (session chair) "Development and application of canine chimeric antigen receptor therapy against a spontaneous model of B cell lymphoma" S3B. Asen Bagashev, Ph.D. "The importance of CD19 exon 2 for surface localization: closing the Ig-like loop" S3C. Ben Chambers "Identification of Hemagglutinin Residues Responsible for H3N2 Antigenic Drift during the 2014-2015 Influenza Season" S3D. Megan Wise "Various forms of CD40L encoded as an immune plasmid adjuvant generate unique anti-HPV DNA vaccine induced responses" S3E. Seth Zost "Prediction of a potential vaccine mismatch during the 2015-2016 influenza season based on monoclonal antibodies isolated from humans in 2011"
11:00 AM – 12:30 PM	Keynote: Douglas Green, Ph.D. Peter C. Doherty Endowed Chair of Immunology St. Jude Children's Research Hospital "LC3-associated phagocytosis: at the interface of aging, inflammation, and innate immunity"
12:30 – 2:20 PM	Lunch and Session IV: Research at the NIH S4A. Tori Yamamoto (session chair) "Fas expression on memory CD8+ T cell subsets augments cellular differentiation and effector function" S4B. Andrea Carpenter Bohrer, Ph.D. "The transcription factor LRF maintains CD8+ T cells' potency and gut homing" S4C. Rachel Gottschalk, Ph.D. "Contextual Control of TLR-induced Responses by Divergent Signaling Thresholds"

2:30 – 3:30 PM	Career Panel: Science from an Editorial Perspective S4A. Avinash Bhandoola, M.B.B.S., Ph.D. Center for Cancer Research, NCI Section Editor, ***The Journal of Immunology*** Editorial Board Member, ***PLOS Biology*** S4B. Thiago Carvalho, Ph.D. Scientific Editor, ***The Journal of Experimental Medicine*** S4C. Ioana Visan, Ph.D. Senior Editor, ***Nature Immunology***
3:30 AM - 5:30 PM	Free Time
5:30 - 7:00 PM	Poster Session + Happy Hour
7:00 - 8:30 PM	Dinner + Awards
8:30 PM – 12:00 AM	Reception

SUNDAY, OCTOBER 25

8:00 - 11:00 AM	Breakfast
11:00 AM	Room Check-out

S1A. "Enhancer regulation of transcriptional bursting parameters revealed by forced chromatin looping"

Caroline Bartman, Chris Hsiung, Arjun Raj, Gerd A. Blobel

Mammalian genes transcribe RNA not continuously but in bursts. Transcriptional output can be modulated through burst frequency or burst size, i.e. the number of RNAs produced per burst. To assess the mechanism by which the b-globin enhancer controls transcription, we combined quantitative single molecule RNA FISH with a recently developed method that enables modulation of enhancer-promoter contacts in the absence of changes to the transcription factor environment or cellular differentiation. We found that both the murine and human b-globin enhancers determine burst frequency but not size. Allelic co-expression of two developmentally distinct human b-like globin genes occurs with comparable burst sizes and with frequencies that are compatible with highly dynamic enhancer-promoter contacts.

S1B. "DNA Double-Strand Breaks Suppress Expression and Activity of the RAG Recombinase in Pre-B Cells"

Megan Fisher, Noah Bloch, Craig Bassing

Developing B and T cells must assemble functional antigen receptor (AgR) genes through the process of V(D)J recombination, in which the Rag1/Rag2 (RAG) endonuclease cleaves widely spaced AgR gene segments and promotes their rearrangement. While crucial for adaptive immunity, this deliberate induction of DNA double-strand breaks (DSBs) poses a risk of oncogenic AgR translocations. Previous work in the Bassing lab showed that RAG-mediated DSBs activate a negative-feedback loop that suppresses Rag expression in pre-B cells. As the presence of multiple DSBs in a cell increases the risk of translocation, I hypothesized that exogenous DSBs would provoke a similar response, to prevent co-existing DSBs at AgR loci and other locations. Accordingly, I have found that exposure to ionizing radiation (IR) activates the DNA-damage response protein ATM to suppress Rag1 and Rag2 transcription in pre-B cells. This is associated with loss of Rag1 protein, which contains the catalytic site of the RAG endonuclease; Rag2 protein persists, but cannot cleave DNA itself. Furthermore, induction of DSBs in a pre-B cell line prevents RAG cleavage at the Igκ locus. Ongoing experiments will determine the mechanism by which DSBs suppress Rag1 and Rag2 transcription. I will also determine whether transcriptional repression of Rag is sufficient to mediate the suppression of RAG activity at Igκ, or whether other factors also play a role.

S1C. "Activating receptor signaling induces MHC I-binding inhibitory receptor expression by natural killer cells"

Jacquelyn Freund, Rebecca M. May, Enjun Yang, Hongchuan Li, Matthew McCullen, Stephen K. Anderson, and Taku Kambayashi

MHC I-binding inhibitory receptors, including those belonging to the Ly49 family, allow natural killer (NK) cells to detect and eliminate virally infected or transformed cells with down-regulated MHC I expression. Ly49 receptors are acquired in a variegated manner during NK cell development leading to a diverse repertoire of NK cells with varying Ly49 expression patterns; however, the mechanism that regulates Ly49 receptor acquisition is undetermined. We recently demonstrated that signals from activating receptors are required for the optimal induction of Ly49 receptors. Mice lacking the adaptor molecule SH2 domain-containing leukocyte protein of 76kD (SLP-76), which display defective NK cell effector responses downstream of activating receptors, harbor significantly decreased proportions of NK cells expressing Ly49 receptors. Surprisingly, only a subset of Ly49 receptors was controlled in an NK cell-intrinsic manner. The adaptor molecules LAT1/LAT2 and ADAP, which represent two separate signaling modules upstream of SLP-76, differentially contributed to Ly49 receptor expression. Mechanistically, SLP-76-derived signals increased the probability of the Ly49G bidirectional Pro1 promoter to transcribe in the forward compared to the reverse direction, which allows mature NK cells to stably express Ly49G. Together, these data support a model where activating receptor signaling in NK cells drives the stable expression of Ly49 receptors during development and explains how the specificity of Ly49 receptors to different MHC haplotypes influences their expression patterns on NK cells.

S1D. "The Role of Trib1 in Granulocyte Development"

Ethan Mack, Sarah Stein, Kelly Rome, Warren Pear

The development of 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, but the developmental stage and mechanism of regulation remain unexplored. We have generated mice conditionally deficient in Trib1 in the hematopoietic compartment using Vav-Cre (VCT1fl/fl). We confirmed that VCT1fl/fl mice have a near complete absence of eosinophils in peripheral tissues, similar to published reports and this correlates with an expansion of neutrophils. In addition, the efficiency of generating eosinophils ex vivo with IL-5 is dramatically reduced compared to wild-type bone marrow. While there is an absence of peripheral eosinophils in Trib1-deficient mice, there are remaining atypical cells in the bone marrow that express a combination of eosinophil and neutrophil antigens in VCT1fl/fl bone marrow. Furthermore, these cells have decreased eosinophil granule protein mRNA and increased neutrophil granule protein mRNA, suggesting that Trib1 controls granulocyte fate decisions. Correlated with this is an increase in C/EBPα p42 protein in VCT1fl/fl neutrophils. Our results suggest that Trib1, possibly through C/EBPα, is a key regulator of eosinophil/neutrophil fate decisions and the atypical cells present in Trib1-deficient bone marrow provide a window into how Trib1 regulates both commitment to and the plasticity of these lineages.

S1E. "A novel lincRNA is a key regulator of group 1 innate lymphoid cell maturation"

Walter Mowel, Sam McCright, Enjun Yang, Makoto Kurachi, Taku Kambayashi, E. John Wherry, Adam Williams, Jorge Henao-Mejia

Epithelial barriers such as the gut, lung, and skin are continuously exposed to the environment and are common sites of inflammation. Innate lymphoid cells (ILCs) are recently described innate cell populations located at these barriers and are critical for defense against a variety of pathogens. Group 1 ILCs include natural killer (NK) cells and ILC1s, and are particularly important for defense against intracellular infections by viruses, bacteria, and parasites. However, the cellular and molecular mechanisms regulating group 1 ILCs are not well understood. Long intergenic noncoding RNAs (lincRNAs) have recently been described as key regulators of gene expression programs in multiple cell types. Importantly, lincRNAs are often expressed in a cell type-specific manner, suggesting that they might regulate specific populations of cells. However, their function in ILC biology is unexplored. Here we describe a novel lincRNA expressed in group 1 ILCs that is critical for their homeostasis. We show that in the context of lincRNA deficiency, while NK cell progenitors are normal, lincRNA-deficient mice are almost completely devoid of ILC1s and mature NK cells in the spleen, liver, lung, and gut due a block in maturation. Furthermore, this phenotype is cell-intrinsic and associated with an overall loss of chromatin accessibility and altered gene expression. Finally, this is specific to group 1 ILCs, as homeostasis of ILC2 and ILC3 populations in the lung and gut were not altered in the context of lincRNA deficiency. Collectively, our data reveals a critical role for a novel lincRNA in ILC1 homeostasis. Allelic co-expression of two developmentally distinct human b-like globin genes occurs with comparable burst sizes and with frequencies that are compatible with highly dynamic enhancer-promoter contacts.

S2A. "When Ndfip1 is away, pathogenic Th17s will play: exploring the role of the ubiquitin cascade protein, Ndfip1, in Th17 abundance and pathogenicity"

Awo Layman, Paula Oliver

T cells may use powerful effector mechanisms such as cytokine release to recruit innate immune cells and enhance the eradication of pathogens. In inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease, there is dysregulation of cytokines, including IL-17A, leading to destructive consequences. The immune system can prevent these destructive consequences by ubiquitination and elimination of effector proteins. Three classes of enzymes, namely: E1 activating, E2 conjugating, and E3 ligase enzymes, work sequentially to ubiquitinate proteins. Ndfip1 is a protein that binds and activates several E3 ligases. Mice lacking

Ndfip1 develop an inflammatory disease characterized by increased numbers of TH17 cells and IL-4 secreting TH2 cells. Little is known about how Ndfip1 regulates TH17 differentiation and function. We have determined that in the absence of Ndfip1, and independent of IL-4, T cells make large amounts of an inflammatory cytokine called IL-17. These Ndfp1-deficient Th17s secrete more IL-17A protein in vitro, and in vivo are more pathogenic in a mouse model of inflammatory bowel disease. In future studies, we hope to carry out proteomic analysis of these Th17 cells in order to determine the substrates regulated by Ndfip1 to limit the abundance and pathogenicity of these Th17 cells.

S2B. "Defining interactions between lymphoid tissue-resident commensal bacteria and the immune system"

Thomas Fung, Matthew R. Hepworth, Nicolas J. Bessman, Dmytro Kobuley, Kelvin Wang, Jeremy Goc, David Artis, Gregory F. Sonnenberg

Dysregulated immune responses against commensal bacteria are associated with the pathogenesis of chronic inflammatory diseases, and can be prevented by maintaining physical separation between commensal bacteria and intestinal immune cells. However, recent reports demonstrate that selective species of commensal bacteria can reside within intestinal-associated lymphoid tissues of healthy mammals. How this unique colonization occurs and functionally influences the immune system is unclear. Here, we demonstrate that lymphoid tissue-resident commensal bacteria (LRC) can colonize murine dendritic cells and modulate cytokine production. In germ-free and antibiotic-treated (ABX) mice, LRCs colonize intestinal-associated lymphoid tissues and induce multiple members of the IL-10 cytokine family, including dendritic cell-derived IL-10 and group 3 innate lymphoid cell (ILC3)-derived IL-22. Notably, IL-10 limits the development of pro-inflammatory Th17 cell responses, and IL-22 production enhances LRC colonization in the steady state. Furthermore, LRC colonization protects mice from lethal intestinal damage in an IL-10-IL-10R-dependent manner. Collectively, our data reveal a novel host-commensal bacteria dialogue whereby selective subsets of commensal bacteria interact with dendritic cells to facilitate tissue-specific responses that are mutually beneficial for both the mammalian host and the microbe.

S2C. "HIV infection induces the expansion of T-bet expressing B lymphocytes"

James J. Knox, M. Anthony Moody, Merlin L. Robb, Michael A. Eller, Mark K. Slifka, E. John Wherry, Michael R. Betts

HIV infection remains a leading cause of infectious disease-related mortality globally. The immunopathology characteristic of the disease affects multiple immune cell subsets and precludes the execution of a protective immune response in most individuals. Examples of B cell dysregulation and dysfunction during HIV infection have been described and likely contribute to the non-protective antibody response observed to HIV and other pathogens. With the recent renewed interest in developing an antibody based vaccine for HIV, understanding the mechanisms promoting either a protective B cell response or B cell dysfunction during the infection is critical. Transcription factors regulate cellular development and control effector functions of immune cells. T-bet is a transcription factor with a well-described role in regulating Th1 cell development. T-bet is also expressed by a subset of B cells, yet its functions are less clear in this cell type. T-bet is known to regulate class switching to anti-viral IgG isotypes in mice and can also regulate chemokine receptor expression. Additionally, T-bet expression in B cells is crucial for controlling a mouse gamma herpes virus infection. Despite its importance during murine viral infections, little is known regarding T-bet expression in human B cells and its role during HIV infection has not been demonstrated. In this study, we sought to investigate the expression of T-bet and identify potential consequences of this expression in B cells during HIV infection.

S2D. "The Activation of Programmed Cell Death During Yersinia Infection Promotes Antibacterial Immunity"

Lance W. Peterson, Naomi H. Philip, Christopher P. Dillon, Douglas R. Green, John Bertin, Peter J. Gough, Igor E. Brodsky

Toll-like receptor (TLR) signaling and the activation of NF-κB- and MAPK-dependent responses are important for the induction of antimicrobial host defenses. Many pathogens interfere with the activation of these defenses through the modulation of innate immune signaling. However, pro-survival and cell death-inducing signals are

coupled downstream of innate immune receptors, such that survival signals prevent cell death in the context of normal inflammatory stimuli. Blockade of key signaling pathways by pathogen virulence factors uncouples this coordinate regulation, resulting in activation of programed cell death. Thus, cell death may act as a conserved host protective mechanism for inducing inflammation in response to pathogens that interfere with innate immune signaling pathways. The YopJ virulence factor of the gram-negative bacterial pathogen Yersinia pseudotuberculosis potently inhibits NF-κB and MAPK signaling, resulting in cell death of innate immune cells via a pathway involving caspase-8 and receptor-interacting serine/threonine kinase 1 (RIPK1). Understanding the role of this cell death response in vivo has previously been confounded by the contribution of many cell death-inducing molecules to cytokine production. However, targeted disruption of RIPK1 kinase activity efficiently blocks Yersinia-induced cell death without impacting cell-intrinsic cytokine production, thus dissociating cell death from other innate immune signaling responses. Through the disruption of RIPK1 kinase activity in vivo, we uncovered a contribution of Yersinia-induced cell death to inflammatory cytokine production and bacterial control. These data suggest that cell death may act as a defensive mechanism for overcoming pathogen inhibition of key signaling pathways and for eliciting a robust anti-pathogen response.

S2E. "Immunostimulatory Defective Viral Genomes from Respiratory Syncytial Virus Promote a Strong Innate Antiviral Response During Infection in Mice and Humans"

Yan Sun, Deepika Jain, Emmanuelle Genoyer, Micah Gilbert, Karla Tapia, Cynthia J. Koziol-White, Reynold A. Panettieri Jr., Richard L. Hodinka, Carolina B. López

Human respiratory syncytial virus (RSV) is a major cause of severe respiratory illness in children and susceptible adults. RSV blocks the development of the innate antiviral immune response and grows to high titers in the respiratory tract until controlled by cellular immunity. Here we demonstrate that immunostimulatory defective viral genomes (iDVGs) that are naturally generated during RSV replication are critical for mounting an effective innate antiviral response and to reduce RSV pathogenicity. In mice, RSV iDVGs stimulated the expression of antiviral genes, restricted viral replication, and prevented weight loss and lung inflammation. In human cells, the antiviral response to RSV iDVGs was dominated by the expression of IFN-λ1 over IFN-β and was driven by rapid intranuclear accumulation of the transcription factor IRF1. RSV iDVGs were detected in respiratory secretions of hospitalized patients, and their amount positively correlated with the level of expression of antiviral genes in the samples. Infection of explanted human lung tissue from different donors revealed that most humans can respond to RSV iDVGs and that the rate of accumulation of iDVGs during infection directly correlates with the quality of the antiviral response. Taken together, our data establishes that iDVGs trigger robust antiviral responses to RSV and provide the first evidence for a critical biological role for naturally occurring iDVGs during a paramyxovirus infection in humans.

S3A. "Development and application of canine chimeric antigen receptor therapy against a spontaneous model of B cell lymphoma"

M. Kazim Panjwani, Jenessa B. Smith, Josephine Gnanandarajah, Daniel J. Powell Jr., Nicola Mason

While chimeric antigen receptor (CAR) therapy has demonstrated potential in treating human malignancies, predictions of safety and efficacy in preclinical mouse models are limited. Canines naturally develop spontaneous malignancies with great similarity to human disease, including B cell lymphomas, and may serve as an improved model to evaluate and optimize CAR immunotherapy.

A method using an artificial antigen presenting cell-based platform was developed for the ex vivo expansion of both CD4+ and CD8+ canine T cells isolated from the peripheral blood of healthy and lymphoma-diseased dogs. These canine T cells can be genetically re-directed either transiently through mRNA electroporation or stably through transduction with lentivirus to express a CAR targeting canine CD20, an antigen present on all known canine B cell lymphomas. Both RNA- and lentivirus-engineered canine CAR T cells produce IFN-γ and lyse target cells in an antigen-specific manner ex vivo, and lentiviral-transduced CAR T cells undergo antigen-specific proliferation to generate functional, antigen-specific daughter CAR T cells.

A twice-relapsed B cell lymphoma patient was treated with RNA-electroporated αcCD20-CD3ζ CAR T cells. Treatment was well-tolerated, and showed a transient anti-tumor effect. Another patient with relapsed, aggressive

B cell lymphoma was treated with lentiviral-transduced αcCD20-CD28-CD3ζ CAR T cells. Disease remained stable for three weeks before progression and treatment with rescue chemotherapy. Collectively, these data demonstrate the feasibility of using the dog as a spontaneous model in which to evaluate and optimize CART therapy as well as provide a potential therapeutic option for canine B cell lymphoma patients.

S3B. "The importance of CD19 exon 2 for surface localization: closing the Ig-like loop"

Asen Bagashev, Elena Sotillo, Glendon Wu, Andrei Thomas-Tikhonenko

CD19 is regarded as a target of choice for B-cell acute lymphoblastic leukemia (B-ALL) immunotherapies. Unfortunately, relapses caused by the loss of the CD19 surface epitopes are a major reason for treatment failure. Recently, we have identified a novel alternatively spliced internalized CD19 isoform lacking exon2 (Δex2 CD19). We aim to identify specific amino acids within exon 2 responsible for proper CD19 surface expression.
We reconstituted full-length, Δex2 CD19 in CD19-null (ΔCD19) derivatives of Nalm6 and 697 B-ALL cell lines generated using CRISPR/Cas9 approach. Additionally, CD19 N-terminal mutations (ΔSP [no signal peptide], N86A, C97A and N86A/C97A) were introduced. Glycosylation was verified using swainsonine (Golgi glycosylation inhibitor) and in vitro de-glycosylation assay.

ΔSP CD19 exhibits a similar surface expression phenotype to Δex2 CD19 with up to 10% of Δex2 CD19 localizing on the plasma membrane. Furthermore, while the N86A substitution in full-length CD19 did not significantly affect its surface localization, C97A mutation fully recapitulated the Δex2 cytosolic phenotype. In fact, the C97A substitution appeared to be retained in the ER, since its electrophoretic mobility was not affected by swainsonine. In contrast, its glycosylation in ER was unperturbed, as evidenced by in vitro de-glycosylation assays.
The immunoglobulin-like loop connecting Cys-38 and Cys-97 of CD19 is required for its surface localization. One possible mechanism is the N-terminus mediated interaction with CD81, which is known to be needed to transport CD19 to the cell surface.

S3C. "Identification of Hemagglutinin Residues Responsible for H3N2 Antigenic Drift during the 2014-2015 Influenza Season"

Ben Chambers, Kaela Parkhouse, Ted Ross, Kevin Alby, Scott Hensley

Influenza vaccines must be updated regularly because influenza viruses continuously acquire mutations in antibody binding sites of hemagglutinin (HA). The majority of H3N2 strains circulating in the Northern Hemisphere during the 2014-2015 season were antigenically mismatched to the A/Texas/50/2012 H3N2 vaccine strain. Recent H3N2 strains possess several new HA mutations, and it is unknown which of these mutations contributed to the 2014-2015 vaccine mismatch. Here, we use reverse genetics to demonstrate that mutations in the HA antigenic site B were primarily responsible for the mismatch. Sera isolated from vaccinated humans and infected ferrets and sheep had reduced hemagglutination inhibition and in vitro neutralization titers against reverse-genetics-derived viruses possessing mutations in the HA antigenic site B. These data provide an antigenic explanation for the low influenza vaccine efficacy observed during the 2014-2015 influenza season. Furthermore, our data support the World Health Organization's decision to update the H3N2 component of future vaccine formulations.

S3D. "Various forms of CD40L encoded as an immune plasmid adjuvant generate unique anti-HPV DNA vaccine induced responses"

Megan Wise, DO Villarreal, L Louis, J Yan, M Morrow, NY Sardesai, DB Weiner

The use of immune plasmid adjuvants encoding cytokine, chemokine or immune modulators to tailor the vaccine induced response is a strength of DNA vaccines. Due to its role in both innate and adaptive immunity, the co-delivery of pCD40L could increase DNA vaccine responses. In its natural form, CD40L can occur as either a surface bound form or a cleaved/solubilized form. Thus, we sought to determine if different forms of pCD40L can influence cellular and humoral responses when co-delivered with a HPV16 DNA vaccine. Mice were immunized with E6 and E7 DNA with or without synthetic optimized plasmids expressing various forms of CD40L. Mice which received the soluble form of CD40L (sCD40L) exhibited significantly higher antigen specific CD8+ T cell responses

including IFN-γ, IL-2 and TNF-α expression. The surface bound form either maintained or blunted the vaccine induced responses compared to vaccine DNA alone. Upon therapeutic tumor challenge, mice immunized with sCD40L displayed significant tumor regression compared to vaccine alone or naïve animals. These results demonstrate the power of using an immune plasmid adjuvant encoding a synthetic optimized sCD40L in a DNA vaccine. Additional studies in other models are necessary for this approach to be considered for possible clinical development.

S3E. "Prediction of a potential vaccine mismatch during the 2015-2016 influenza season based on monoclonal antibodies isolated from humans in 2011"

Seth Zost, Amy Davis, Trini Ochoa, Patrick C. Wilson, Scott E. Hensley

Influenza A viruses pose a serious public health threat, infecting millions of people annually and causing considerable morbidity and mortality. Infection or vaccination induces antibody responses against the viral protein hemagglutinin (HA) that protect individuals from re-infection, but influenza viruses escape human antibody responses through the acquisition of mutations in HA that prevent the binding of pre-existing antibodies. This process of immune evasion is known as antigenic drift. In order for the vaccine to induce protective antibodies, it is essential that the vaccine and circulating strains be antigenically matched. This year, the WHO recommended the inclusion of Switzerland/9715293/2013 (Switz/2013) strain as the updated H3N2 component of the vaccine. This strain, however, belongs to a different clade compared with strains that dominated circulation during the most recent flu season. This raises the question of whether the Switz/2013 strain will provide effective protection against circulating H3N2 strains. Here, we show that human anti-HA monoclonal antibodies (mAbs) isolated from individuals following following vaccination with a 2010 influenza vaccine can define the antigenic differences between Switz/2013 and other strains. Additionally, serum from ferrets infected with the Switz/2013 vaccine strain exhibits reduced reactivity to a virus from the current dominant clade. This result suggests a possible antigenic mismatch between the vaccine strain and H3N2 strains anticipated to circulate during the 2015-2016 season, and illustrates the potential utility of using human mAbs in viral surveillance and vaccine strain selection.

S4A. "Fas expression on memory CD8+ T cell subsets augments cellular differentiation and effector function"

Tori N. Yamamoto, Anthony J. Leonardi, Hui Liu, Ena Wang, Luca Gattinoni,
Anthony C. Cruz, Claudia Ouyang, Richard M. Siegel, Nicholas P. Restifo, and Christopher A. Klebanoff

Memory CD8+ T cells (T_{Mem}) provide lifelong protection against intracellular pathogens and cancer. Despite phenotypic and functional heterogeneity, all T_{Mem} subsets express Fas, a tumor necrosis family receptor superfamily member conventionally known as a death receptor. Because Fas can mediate non-death signaling in other cell types, we sought to elucidate the role of Fas signaling in defined T_{Mem} subsets, including T stem cell memory (T_{SCM}), T central memory (T_{CM}), and T effector memory (T_{EM}). We found that augmenting Fas signaling in T_{SCM} using an oligomerized form of its ligand FasL enhanced cellular differentiation and a loss in IL-2 secretion. Conversely, antibody blockade (aFasL) of Fas signaling in T_{CM} retarded cellular differentiation both phenotypically and functionally. To genetically disentangle the pro-apoptotic and pro-differentiation functions of Fas, we used a mutant Fas lacking a transmembrane cysteine residue (FasC194V) that is unable to undergo S-palmitoylation and aggregate in lipid rafts. Using transgenic mice expressing FasC194V on a Fas-deficient lpr background, we found that FasC194V T_{Mem} still undergo cellular differentiation in the absence of death signaling. In vivo, T_{Mem} expanded with aFasL showed greater expansion, on-target immunity and withheld differentiation. Additionally, T_{Mem} expanded with aFasL and genetically engineered with an anti-CD19 chimeric antigen receptor (CAR) exhibited enhanced CAR expression, reduced differentiation, and augmented anti-lymphoma 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.

S4B. "The transcription factor LRF maintains CD8+ T cells' potency and gut homing"

Andrea C. Carpenter, Jasmin Herz, Dorian B. McGavern, and Rémy Bosselut

CD8+ cytotoxic T cells are important for clearance of infectious pathogens. As such elucidating the transcriptional circuitries that control development and function of these and CD4+ helper T cells is critical for understanding pathways that guide T cell protective immunity. We recently showed that the transcription factors Thpok and LRF redundantly promoted helper T cell effector differentiation. Both are members of the BTB/POZ family, but while Thpok is normally expressed in CD4+ helper cells, LRF is expressed in both helper cells and CD8+ cytotoxic cells. Consequently, we inquired if LRF alone contributed to cytotoxic effector T cell differentiation or function. LRF was not needed for the positive selection of CD8+ T cells. However, we found that LRF controls CD8+ T cell effector functions. LRF-deficient CD8+ T cells are unable to mount proper responses to acute lymphocytic choriomeningitis virus (LCMV). Compared to wild-type mice, mutant mice had fewer LCMV-specific CD8+ cells and among those there was a significant reduction in cytokine producing cells. Furthermore, LRF-deficient cells fail to respond to rechallenge. Accordingly, we asked whether LRF- deficient CD8+ tissue-resident memory cells were maintained and notably found that LRF-deficient cells were unable to occupy the small intestine. We are currently working on the mechanisms through which LRF supports effector, memory, and tissue memory cells. These observations suggest a previously unanticipated role for LRF in maintaining global CD8+ T cell protective immunity.

S4C. "Contextual Control of TLR-induced Responses by Divergent Signaling Thresholds"

Rachel Gottschalk, Ronald Germain

The innate immune system generates context-specific responses to microbial products, distinguishing steady-state stimuli from those of invasive pathogens, but we lack a mechanistic understanding of the signaling logic that limits inflammatory cytokine production upon exposure to non-dangerous inputs. Using quantitative assays and computational modeling, we found multiple thresholds for signaling pathways dictating stimulus-dependent functionality in TLR4-stimulated mouse and human macrophages. While inflammatory responses were controlled by switch-like MAPK activation, NF-κB activity occurred at ligand concentrations below the MAPK-mediated inflammation threshold, resulting in restricted gene expression and macrophage priming for an enhanced response to subsequent stimulation. Our study thus reveals a tightly regulated low-noise, robust response system, in which NF-κB-dependent 'danger discrimination' is facilitated by strict negative regulation of MAPK activity.

The use of immune plasmid adjuvants encoding cytokine, chemokine or immune modulators to tailor the vaccine induced response is a strength of DNA vaccines. Due to its role in both innate and adaptive immunity, the co-delivery of pCD40L could increase DNA vaccine responses. In its natural form, CD40L can occur as either a surface bound form or a cleaved/solubilized form. Thus, we sought to determine if different forms of pCD40L can influence cellular and humoral responses when co-delivered with a HPV16 DNA vaccine. Mice were immunized with E6 and E7 DNA with or without synthetic optimized plasmids expressing various forms of CD40L. Mice which received the soluble form of CD40L (sCD40L) exhibited significantly higher antigen specific CD8+ T cell responses

P1. "Early onset and persistence of metabolic alterations in exhausted T-cells is controlled by PD-1"

Bertram Bengsch

Dynamic reprogramming of metabolism is essential for T-cell effector function and formation of memory. However, regulation of cellular metabolism in exhausted T-cells in chronic infections and cancer is poorly understood. Here we found that within the first week of chronic LCMV infection, virus-specific CD8 T-cells are already unable to match the bioenergetic demands of effector CD8 T-cells generated during acutely resolving LCMV infection. Suppression of T-cell bioenergetics involves restriction of glucose uptake and utilization, despite the up-regulation of multiple other metabolic pathways. The inhibitory receptor PD-1 controlled glycolysis as well as mitochondrial mass and quality in the presence of persisting mTOR signaling. The suppression of glycolysis and mitochondrial metabolism in exhausted T-cells persist into established chronic infection. However, therapeutic reinvigoration of exhausted T-cells by PD-L1 blockade reprogrammed the metabolism of PD-1int subset exhausted T-cells but not the PD-1Hi terminal subset. These data highlight a key metabolic control event early in T-cell exhaustion that precedes major transcriptional changes. Our findings also suggest that reprogramming of T-cell metabolism by checkpoint blockade may be involved in therapeutic outcomes.

P2. "Antibiotics Treatment Alters TLR9 Responses in Vivo"

Chhanda Biswas

The gut microbiota affects inflammation and immunity both locally and systemically. This raises the question of whether the microbiota affects inflammatory processes in our TLR9-driven model of cytokine storm. Here we examine the impact of changes in microbiota induced by antibiotics on TLR9 responses. The C57bl/6J mice were treated with antibiotics mixture (ABX) of ampicillin, neomycicn, vancomycin and metronidazole (1 g/liter) supplemented in drinking water. After three weeks of ABX treatment the mice received five-repeated intraperitoneal injection of CpG alternate day while the ABX was continued. When compared with the control mice, which received only water, the CpG-induced splenomegaly and hepatomegaly, was profoundly reduced in ABX treated mice. This further correlated with a reduced infiltration of inflammatory monocytes in spleen and liver and reduced levels of serum proinflammatory cytokines; IL-10, IL-6, IL-12 and IFN-γ. This suggested that ABX provides protection from TLR9-driven inflammation. To test this hypothesis, we ABX treated mice and then tested TLR9 responses in vitro from cells from various organs. In both spleen and bone marrow, numbers of TLR9 responsive cells and per cell TLR9 induced IL-12 was reduced. Taken together, these data suggest that an intact 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.

P3. "Regulation of CD19 exon 2 inclusion in B-lymphoid cells by splicing factors and epigenetic marks"

Kathryn Black

CD19 is a B-cell surface marker and a target for immunotherapies against B-cell malignancies, including pediatric B-ALL. Immunotherapies targeting CD19 are widely successful, but relapses lacking the CD19 epitope still occur (Maude, 2014). We discovered that alternative splicing of CD19 exon 2 is responsible for the loss of extracellular domains, causing resistance to therapy (Sotillo, 2015). Here we investigate the molecular mechanism of CD19 exon 2 skipping.

Sequence-based algorithm AVISPA (Barash, 2013) predicts splicing factors (SF) to bind RNA. RNA crosslink immunoprecipitation in nuclear lysates from Nalm-6 B-ALL cells tests the direct binding to exon 2 of 9 AVISPA-

predicted SFs and 6 SFs commonly involved in exon skipping. We identified SRSF3, hnRNP-A, and hnRNP-C as CD19 exon 2-bound proteins. SiRNA knockdown experiments revealed that downregulation of SRSF3, but not hnRNP-C, increases the frequency of exon 2 skipping in a dose dependent manner, suggesting SRSF3 promotes the inclusion of exon 2. To further validate the role of SRSF3 in CD19 splicing we mined the publicly available GSE52834 dataset where 22 RNA binding proteins were knocked down in a lymphoblastoid cell line and only SRSF3 knockdown caused an increase in exon 2 skipping, suggesting that SRSF3 is the key regulator of CD19 splicing.

SRSF3 interacts with PSIP1, which "reads" H3K36me3 (Pradeepa, 2012). Exonic regions in genomic DNA are enriched for H3K36me3, and knockdown of Setd2, the H3K36 methyltransferase, alters exon inclusion (Luco, 2010; Brown, 2012; Hnilicova, 2011). Thus, we are investigating the connection between H3K36me3 and alternative splicing of CD19.

P4. "Shared VH1-46 antibody gene usage in pemphigus vulgaris predicts antibody cross-reactivity to desmoglein 3 and rotavirus VP6"

Michael Cho

Pemphigus vulgaris (PV) is a skin blistering disease caused by autoantibodies (autoAbs) to desmoglein 3 (Dsg3). We have identified VH1-46 gene usage in the anti-Dsg3 repertoire among 4 different patients via high throughput B cell receptor (BCR) cloning. These VH1-46 BCRs also require few to no acidic amino acid mutations to bind Dsg3. Shared VH1-46 gene usage also occurs in the B cell response to the rotavirus protein VP6 and similarly, few somatic mutations are required for VP6 reactivity. We investigated whether Dsg3-reactive VH1-46 BCRs are cross-reactive to VP6 and may explain the tolerance of these autoreactive clones. We screened ~108 IgM B cell clones from a PV patient against Dsg3 and VP6, and demonstrated VH1-46 enrichment in 7/13 clonal families. ELISA and immunofluorescence studies confirm that of the 6 VH1-46 clonotypes able to be purified as soluble Abs, 5 cross-reacted to VP6 and Dsg3, while 1 bound VP6 and only weakly bound Dsg3. Additionally, 3 of 5 VH1-46 anti-Dsg3 IgG clonotypes previously isolated from 3 different PV patients cross-reacted to VP6 in either their somatically mutated or unmutated form. A subset of cross-reactive Abs inhibited rotavirus transcription similar to an Ab isolated from an individual after rotavirus infection that blocks RNA extrusion through VP6. Our data suggest that a VH1-46 B cell response to rotavirus may be an initiating event that develops into an autoimmune response to Dsg3 in susceptible individuals.

P5. "Exploring the role of Type 1 IFN during Toxoplasma gondii infection"

Lucas Dawson

The protective immune responses against Toxoplasma gondii depends on a robust TH1 immune response, during which IFNg induces intracellular killing mechanisms in both hematopoietic and non-hematopoietic infected cells in a STAT1 dependent manner. However, these immune mechanisms are not able to achieve sterile clearance of the parasite, and T. gondii is able to evade the host immune response and establish a chronic infection that can last for the host's lifetime. Similar to IFNg, Type 1 IFNs are also able to signal through STAT1 and induce intracellular killing mechanisms, however the importance of Type 1 IFNs during T. gondii infection is not well understood. To determine the role of Type 1 IFNs during murine toxoplasmosis, we performed infections of IFNAR1 KO mice. Surprisingly we found that these mice were unable to survive chronic infection, and displayed increased parasite burdens compared to WT controls. This suggested a breakdown in the protective host immune response, and indeed we observed that IFNAR1 KO mice have reduced IL-12 production and defective T cell responses following infection. Additionally, preliminary data suggests that IFNAR1 KO mice have reduced accumulation of molecules important for intracellular parasite killing around parasite cysts during chronic infections. Future work will use IFNAR1 flox and lineage specific Cre mice to determine what cells require IFNAR1 signaling to generate protective immune responses and control parasite burdens during toxoplasmosis.

P6. "IL-27 promotes T cell expression of inhibitory receptors"

Jonathan DeLong

Inhibitory receptors are a diverse group of proteins expressed by T cells that help limit T cell proliferation and cytokine secretion and are linked with the phenomena of “immune exhaustion”. Recent success of biological therapeutics targeting CTLA-4 for rheumatoid arthritis and PD-1 and CTLA-4 for cancers suggest that modulation of these molecules might ameliorate a wide range of diseases. TCR stimulation is known to drive expression of these molecules, but the role of cytokines in this process is only beginning to be understood. In 2012, studies from this laboratory helped establish that the cytokine IL-27 induces PD-L1 on CD4+ T cells. IL-27 is an IL-6/IL-12 family cytokine that is important in preventing immune hyperactivity during infection. Transcriptional profiling suggested that IL-27 drives expression of multiple inhibitory receptors, prompting further investigation. I have found that IL-27 drives expression of LAG-3, TIGIT, CTLA-4 and PD-L1 by T cells in vitro. This upregulation can be dependent (e.g. CTLA-4) or independent (e.g. PD-L1) of TCR stimulation. Some of these receptors downregulate DC functions and our preliminary data suggest that IL-27 enhances the ability of Tregs to suppress DC maturation. I am using infection with the protozoan parasite Toxoplasma gondii to examine the in vivo relevance of these findings. IL-27 is required for controlling immunopathology during toxoplasmosis and I have found that IL-27 contributes to inhibitory receptor expression during infection. Future studies will examine the role of these inhibitory receptors in suppressing immunopathology in this model.

P7. “Ndfip1 restrains Treg function and homeostasis to prevent inflammation at mucosal surfaces”

Guoping Deng

Regulatory T cells (Tregs) are a subpopulation of T cells that suppress immune function to prevent autoimmune disease and prevent collateral damage during infection. Understanding how Treg cells acquire and execute their suppressive function is relevant for the study of disease pathogenesis and for modulating activity for therapeutic effect. The ubiquitin system is known to regulate the differentiation and function of conventional T (Tconv) cells. Our lab has shown that the E3 ubiquitin ligase Itch, and its co-activator Ndfip1, are required for inducible Treg (iTreg) differentiation. Our new data show that Ndfip1 also regulates the function and homeostasis of Treg cells even after lineage commitment.

We generated Ndfip1fl/flFoxp3-Cre mice lacking Ndfip1 only in Treg cells. By 12 weeks of age, Ndfip1fl/flFoxp3-Cre mice developed overt auto-inflammation at mucosal barrier surfaces, namely skin and lung. Ndfip1fl/flFoxp3-Cre Tconv cells were highly activated and making cytokines characteristic of TH1, TH2 and TH17 cell subsets. In line with this, serum antibody levels were elevated significantly. Importantly, this was not because Treg cell frequencies and numbers were reduced in Ndfip1fl/flFoxp3-Cre mice, nor were they defective in their ability to migrate into tissues. Rather, Ndfip1fl/flFoxp3-Cre Treg cells showed altered profiles of Treg subsets and expression of Treg-associated protein markers. Our Co-IP pull-down and proteomics analysis suggested that signaling from TCR- and Jak1-related pathways are potentially involved in these phenotypes. Together, our data demonstrate that Ndfip1 is indispensable for the maintenance of Treg homeostasis and prevention of inflammation at mucosal barrier surfaces.

P8. "New Insights into the Complex Regulation of the Glycolytic Pathway in Lactococcus lactis"

Sepideh Dolatshahi

This study uses computational modeling based on in vivo NMR data to decipher the complex coordination of regulatory signals of glycolysis in Lactococcus lactis under aerobic and anaerobic conditions.

P9. "Identification of unique and conserved salvage pathways among human CD4 subsets in response to low glucose"

Christopher Ecker

T cells play a crucial role in many anti-tumor responses in vivo. Recent clinical trials focusing on adoptive transfer of tumor-specific T cells have been successful in types of leukemia. However, the success rate of these 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 perform glycolysis, proliferate, and produce effector cytokines. However, the cellular salvage pathways that allow T cells to survive and function during nutrient stress, and how distinct T cell subsets may utilize these pathways differently is not understood. Our laboratory demonstrated that bulk CD4+ T-cells adapt to low glucose levels by increasing oxidative phosphorylation. We have observed that exogenous fatty acids are essential for survival of CD4+ T cells grown in low glucose. Pharmalogical inhibition of fatty acid oxidation by etomoxir was sufficient in inhibiting the increase in oxidative phosphorylation and decreasing expansion in cells only grown in low glucose. We have found that naïve and central memory cells mimic the responses to low glucose we observed in bulk T cells. However effector memory T cells do not adapt their metabolism, and actually maintain IFNy production in low glucose. Understanding how T cells adapt to the limiting nutrients in the tumor microenvironment may be critical for augmenting traditional adoptive T cell therapy.

P10. "Desmoglein 3 chimeric autoantibody receptor (CAAR) T cells: a novel strategy for immunotherapy of pemphigus vulgaris"

Christoph Ellebrecht

Pemphigus vulgaris (PV) is a blistering skin disease caused by autoantibodies (autoAbs) to desmoglein (Dsg) 3. PV therapy relies on general immunosuppression, risking severe infection with chronic use. Recently, chimeric antigen receptor (CAR) T cells have revolutionized immunotherapy. To evaluate this potent approach for PV, we engineered a series of Dsg3 chimeric autoAb receptors (CAARs), consisting of various Dsg3 extracellular (EC) domains fused to either T or NK receptor signaling domains. In vitro, Dsg3 CAAR T cells specifically kill target cells expressing surface anti-Dsg3 EC1, EC2, or EC3 autoAbs, with the most broad and potent cytolysis by the Dsg3 EC1-4 CAAR using the T cell CD137CD3ζ signaling domain ($p=0.0001$). Dsg3 CAAR T cells do not kill Fcreceptor+ cells coated with Dsg3 autoAbs or keratinocytes whose surface proteins might interact with the Dsg3 CAAR.

TIRF microscopy reveals that autoAb binding by the Dsg3 CAAR forms supramolecular activation complexes, with segregation of the CAAR away from CD45, a regulator of T cell receptor activation. In a preclinical PV NSG mouse model, bioluminescent AK23 (anti-EC1 surface IgG+) hybridoma cells were efficiently killed by Dsg3 CAAR ($p=0.0015$), resulting in prolonged survival of CAAR-treated versus control mice ($p=0.016$) without skin toxicity. CAAR T cells represent a novel strategy for PV therapy that specifically targets autoantigen-specific B cells and that could readily be applied to other autoAb-mediated diseases.

P11. "Directing iNKT Cells To Tumor Targets Via CAbs"

Gabrielle Ferry

Invariant natural killer T (iNKT) cells are innate-like lymphocytes that share phenotypic characteristics with both T and NK cells but are restricted by CD1d, a non-classical MHC class I-like molecule that presents glycolipid antigens (GAg). We and others previously showed that iNKT cells participate directly and indirectly in anti-tumor responses. We recently developed a strategy to augment the anti-tumor activity of iNKT cells against CD1d+ and CD1d- tumors. In this strategy, we conjugated antibodies specific for tumor-specific antigens (TSAs) to CD1d molecules displaying iNKT cell-stimulating glycolipid antigens (CD1d-GAg). We hypothesize that these CD1d-GAg–-anti-TSA-mAb (“CAb”) conjugates will concentrate within TSA-expressing tumors and activate iNKT cells in situ. Activated iNKT cells would then exert direct cytotoxicity, facilitate ensuing anti-tumor responses via

production of pro-inflammatory cytokines, and/or potentially alter the tumor microenvironment. In support of this hypothesis, we demonstrate that TSA-targeted CAb increases in vitro lysis of human and murine tumors by effector cell populations enriched in iNKT and NK cells. Additionally, we observe rapid intracellular production of IFN-γ and upregulation of granzyme B in iNKT and NK cells within hours of CAb administration in vivo. Finally, our preliminary data also suggest that the use of TSA-specific (but not control) CAb restricts the growth of exogenously-administered tumors in lymphocyte-deficient mice that are repleted with small numbers of iNKT and NK cells. Our ongoing and future studies will help clarify whether CAb and iNKT-mediated anti-tumor responses could be harnessed for use in platforms of immunotherapy.

P12. "The roles of Itch and Ndfip1 in regulating macrophage bacterial response"

Natania Field

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. The formation of ubiquitin chains on protein substrates is crucial in regulating macrophage signaling cascades. This post-translational modification can target a protein substrate for either downstream signaling or for degradation. E3 ubiquitin ligases, such as Itch, are required to facilitate the formation of these chains. Itch exists in an autoinhibited state, and can be activated by Nedd4-family interacting protein 1 (Ndfip1). It was recently discovered that Itch and its activator Ndfip1 limit T cell responses. In contrast, the roles of these proteins in macrophage function, and bacterial clearance, is less well understood.

Macrophages can contribute to bacterial clearance through producing inflammatory cytokines and by engulfing and killing bacteria directly. Itch has been implicated in both of these functions. It is unknown how Itch influences macrophage response to an in vivo bacterial infection, and whether Ndfip1 plays a role in one or both of these contexts. I hypothesize that Ndfip1 activates Itch in macrophages to limit inflammatory cytokine production in response to TLR4 stimulation, and attenuates the macrophage response to gram-negative bacterial pathogens. I will test this hypothesis using in vivo and biochemical approaches. This information is crucial in understanding how a potential Itch-targeted drug that acts as an Ndfip1 mimetic could affect susceptibility to bacterial lung infection.

P13. "Interferon-Induced Protein With Tetratricopeptide Repeats 1 (IFIT1) alters the course of paramyxovirus infection"

Jennifer Grier

During rapidly replicating RNA virus infections, the viral polymerase becomes error-prone and generates shortened defective viral genomes (DVGs). These DVGs induce a potent antiviral immune response characterized by production of interferon and up-regulation of Interferon Stimulated Genes (ISGs). One such ISG is IFIT1, which binds to viral RNA lacking a 2'O-methyl cap to block translation and potentially alter transcription of RNA viruses. Investigation of host factors that contribute to DVG-triggered antiviral immunity is important for understanding innate responses and protective pathways following infection with viruses such as Measles or Respiratory Syncytial Virus, which naturally produce DVGs. Sendai Virus (SeV), is a (-)ssRNA virus that serves as a model to study mechanisms of virus-host interactions in the presence or absence of DVGs. Following SeV infection with DVGs, IFIT1 mRNA is quickly upregulated to a very high level, yet the functional significance of this is unclear as SeV mRNA is 2'O-methyl-capped. Mouse and human cell lines lacking IFIT1 protein demonstrate diminished cell numbers following a High-DVG paramyxovirus infection. This is not due to increased viral load as the knockout cell lines generated lower levels of viral products per cell compared to wild type controls. Dysregulation of ATP production was also observed in IFIT1 -/- human lung epithelial cells, primarily at late times post infection when newly-generated DVGs are present, suggesting an effect on cellular metabolism, potentially via changes to mitochondria which are associated with intracellular RNA-sensing pathways. Thus, IFIT1 alters the course of paramyxovirus infection but the specific mechanisms remain unclear.

P14. "A role for T-bet in coordinating T cell activation"

Gretchen Harms Pritchard

The T-box transcription factor T-bet is perhaps most prominently known as a master regulator of Th1 differentiation and IFNγ production. Recent work from our laboratory has identified a novel role for this transcription factor in coordinating the effector T cell responses necessary to control the intracellular parasite Toxoplasma gondii in peripheral tissues, and suggests that T-bet may function in a diverse array of immunological processes. The integrin CD11a is involved in cell:cell interactions during T cell priming and activation. Here, we show that T-bet is required for optimal upregulation of CD11a and ICAM-binding, an observation that suggests that T-bet is required for optimal cell:cell interactions during T cell activation. Support for this idea is provided by studies which showed that in mice challenged with T. gondii, the absence of T-bet or CD11a blockade results in a similar reduction in T cell responses. Thus, these findings highlight the importance of these proteins for in vivo T cell responses and suggest a role for T-bet in the coordination of the very early events in T cell activation that are necessary for optimal CD8+ T cell responses.

P15. "Delineating the role of pioneer transcription factors in lymphoid cells"

John Johnson

The dogma of cell development is that progenitor cells have open, accessible genomes that become condensed and silent during differentiation to commit a cell to a particular lineage. “Pioneer transcription factors" act contrary to this dogma and engage silent genomic sites in progenitor cells, open these sites, and initiate developmental changes. The transcription factors that initiate these regulatory events in lymphoid cells have not been previously characterized. Thus, we set out to investigate the role of pioneer transcription factors in lymphoid development and to characterize the histone modifications that correspond to their activity. Our systematic analysis of genome-wide chromatin accessibility in hematopoietic and lymphoid cells, using ATAC-seq, revealed genomic regions that are initially closed in hematopoietic cells. These sites later become accessible in terminally differentiated lymphoid cells, indicating pioneer transcription factor activity. Analysis of the histone modifications at these genomic regions, obtained from ChIP-seq, revealed sequential poising of these enhancers until the final cell product is reached. After terminal differentiation, these poised modifications are removed from genomic regions corresponding to opposite lymphoid lineages and seal the developmental fate of the cell. Motif analysis of transcription factor binding sites revealed an enrichment of motifs for certain transcription factors at these genomic regions. For example, in the genomic regions of B cells we found an enrichment of motifs corresponding to the ETS transcription factor PU.1. We will further investigate these transcription factors as we continue to uncover the role of transcription factors that act as "pioneers" during lymphoid cell development.

P16. "A long non-coding RNA regulates myeloid cell turnover"

Jonathan Kotzin

Large intergenic non-coding RNAs (lincRNAs) are a class of non-coding RNA that have been reported to play crucial roles in a variety of processes and diseases. Myeloid cells are potent but typically short-lived mediators of innate immunity and inflammatory responses. As such, alterations in myeloid cell survival have been closely associated with immunodeficiency, autoimmunity, and malignancy. We hypothesize that there are lincRNAs specifically expressed by myeloid cells that are critical in regulating the turnover of these cells.

Using high throughput sequencing we identified a candidate lincRNA, Linc30, which is highly and specifically expressed by eosinophils, neutrophils, and monocytes. Linc30 has a direct human homologue, a conserved pattern of expression in humans, and an association with human allergic disease through GWAS. Using the CRISPR/Cas9 system to generate a knockout mouse model, we found that Linc30 is required for the development of eosinophils, neutrophils, and inflammatory monocytes; however, the respective progenitors of these cells remain intact. Linc30-deficient mice also have impaired functional immunity and are highly susceptible to Listeria Monocytogenes infection.

LincRNAs are well known to regulate the expression of neighboring genes, and Linc30 is located ~150kb upstream of Bcl2l11 (Bim), a potent pro-apoptotic factor. We observed that Bim expression is dysregulated in cis in Linc30-deficient myeloid cells, and these cells are prone to programmed cell death. Together, our studies provide insight into the roles of lincRNAs in the regulation of hematopoiesis. Additionally, lincRNAs and the factors that regulate them may represent potential therapeutic targets to modulate myeloid cell function.

P17. "A Role for Myeloid Cells in Regulating Metastasis in Pancreatic Carcinoma"

Jae Lee

Pancreatic ductal adenocarcinoma (PDAC) is the fourth-leading cause of cancer-related deaths in the United States, and the vast majority of PDAC patients present with metastatic disease with the liver representing the most common site of disease spread. During tumor development, primary tumor cells can secrete factors that precondition the liver for metastasis. In this process, the liver becomes a “pro-metastatic niche” that is more receptive to tumor cell seeding. Myeloid cells are a prominent component of this niche, yet their role in regulating PDAC metastasis is unknown. In this project, we have studied a role for myeloid cells in establishing a pro-metastatic niche. We hypothesize that myeloid cells are a critical determinant of metastasis and acquire pro-tumor properties early during tumor development. In our preliminary studies, we have found that myeloid cells accumulate within the liver in a genetically engineered murine model of PDAC. These myeloid cells appear activated via the Signal Transducer and Activator of Transcription 3 (STAT3) signaling pathway, which may play a key role in inducing myeloid cells with pro-tumor properties. In order to demonstrate that tumor cells are driving changes in the liver microenvironment, we have established an intraperitoneal model of PDAC. Using this model, we have found that intraperitoneal implantation of PDAC cells leads to an increased number of STAT3-activated myeloid cells in the liver. Our project will improve the understanding of pathways regulating metastasis and may identify novel therapeutic strategies for the treatment of PDAC.

P18. "Improving CAR Therapy for HIV to Achieve Durable Control Over Virus Replication"

Rachel Leibman

The cytotoxic T-lymphocyte (CTL) response of HIV-1 patients is severely compromised during chronic infection. Attempts to redirect patients’ CTLs to target HIV and restore HIV-specific cytotoxic responses have utilized HIV-specific T cell receptors (TCRs) and chimeric antigen receptors (CARs) with little success. CARs consist of an extracellular antigen binding domain fused to intracellular T-cell activation domains. CARs are an attractive adoptive therapy tool because of their ability to bind and kill HIV-infected cells independently of MHC expression and antigen presentation, both of which may be decreased or impaired in HIV-infection. CARs that contain the CD4 extracellular domain kill HIV-infected target cells after binding the HIV-1 Envelope glycoprotein expressed at the plasma membrane. However, the high level of CD4 on CAR-transduced cells promotes infection of these cells. Infection of CAR+ cells can be reduced with GP41-based mimetic peptides. When fused to the CXCR4 coreceptor, these peptides are expressed at the cell surface in a location desirable for virus fusion. Preventing infection may improve control over HIV-1 in vivo and result in durable suppression of virus replication.

P19. "Skin-mediated control of systemic regulatory T cell numbers and protection against type I diabetes"

Theresa Leichner

Regulatory T cells (Tregs) are a subset of CD4+ T cells with suppressive function. Tregs are critical in limiting autoimmunity and increasing Treg numbers can be beneficial in the treatment of various inflammatory disorders. Here, we provide evidence that the skin can exert strong systemic effects on Treg numbers by producing the cytokine thymic stromal lymphopoietin (TSLP) in response to topical administration of the Vitamin D3 analog MC903. A 2-3 fold increase in the proportion (out of all CD4+ T cells) and absolute number of Tregs was observed in the blood, lymph nodes, and spleen of mice treated topically but not systemically with MC903. The increase in Treg numbers was dependent on TSLP-R signaling but not on Vitamin D receptor signaling in hematopoietic cells. However, TSLP-R expression by Tregs themselves was not required for the proliferation of Tregs induced by topical

MC903 treatment. Rather, TSLP likely promoted Treg proliferation by affecting the DC/Treg interaction, as TSLP alone or in combination with IL-2 induced the proliferation of Tregs co-cultured with DCs. To test whether these effects of MC903 could influence progression of an autoimmune disorder, non-obese diabetic (NOD) mice were treated topically with MC903. Treatment with MC903 compared to vehicle significantly lowered the incidence of diabetes from 100% to 40% ($p<0.05$). Together, these data demonstrate that the skin has the remarkable potential to control systemic immune responses and that topical MC903 treatment could serve as a novel strategy to induce systemic immunomodulation in autoimmune diseases.

P20. "A novel Mendelian disease reveals an essential role for GIMAP5 and GIMAP6 in human immune homeostasis"

Alex Leney-Greene

We have identified a cohort of patients suffering from a novel Mendelian autoimmune disease. We identified causative mutations GIMAP5 and GIMAP6, and aim to understand their role in human immunity.

P21. "Uncovering the hidden characteristics of original antigenic sin antibodies"

Susanne Linderman

Circulating influenza viruses are constantly accumulating mutations that change their antigenic characteristics and most humans are therefore exposed to many distinct influenza strains throughout their lives. Antibodies (Abs) elicited by influenza viruses often bind with a high affinity to past influenza virus strains, but paradoxically, do not bind to the viral strain actually eliciting the response. This phenomena is called 'original antigenic sin' (OAS), since this can occur at the expense of generating new de novo Ab responses. Using a mouse model we find that OAS and non-OAS Abs target the same general region of the influenza hemagglutinin protein but that the majority of Abs recalled by an antigenically distinct strain have fine specificities that differ from those of Abs recalled by a repeat exposure to the older strain. We also find that somatic hypermutation can convert OAS Abs into cross-reactive Abs capable of recognizing multiple influenza virus strains. Surprisingly, although OAS Abs bound with very low affinities, they were able to effectively neutralize an antigenically drifted viral strain following passive transfer in vivo. Taken together, our data indicate that OAS Abs share some level of cross-reactivity between priming and recall viral strains and that these Abs can be beneficial when recalled into secondary immune responses.

P22. "The ubiquitin ligase Itch controls B cell activation and antibody production"

Emily Moser

Mice deficient in the ubiquitin ligase Itch spontaneously develop lethal systemic autoimmune disease characterized by aberrant CD4+ T cell differentiation to the Th2 lineage and excess serum antibody production. Although the role of Itch in CD4+ T cells has been well studied, the mechanisms by which Itch regulates B cells are not well known. We sought to determine if Itch deficiency affected B cell function independently from the excess Th2 cells present in Itch-/- mice. To this end, we examined serum antibodies and B cell populations in mice doubly deficient in Itch and IL4 (Itch-/-IL4-/-). We found that although serum antibodies were not increased, numbers of activated splenic B cells were significantly elevated in the Itch-/-IL4-/- mice compared to controls. Additionally, NP-ova immunization of mixed bone marrow chimeric mice revealed that NP-specific B cells and antibody secreting cells were preferentially derived from the Itch-deficient B cells, suggesting that Itch controlled B cell function in a cell-intrinsic manner. Finally, to determine which activation pathways were controlled by Itch in B cells, we measured proliferation of mature naïve B cells after activation in vitro, and we found that Itch-/-IL4-/- B cells proliferated more than controls in response to CpG, but not anti-IgM or LPS. Current efforts are underway to determine the mechanism(s) by which Itch controls B cell activation and antibody production to regulate protective immunity and limit autoimmunity.

P23. "Quantitative proteomics predicts functional consequences of ubiquitylation events during T cell stimulation"

Claire O'Leary

The post-translational attachment of ubiquitin to cellular proteins can alter their function and half-life and thus profoundly impact signaling and cell fate decisions. Not surprisingly, the consequences of protein ubiquitylation are highly context dependent. New quantitative proteomics platforms are currently being developed that have the potential to reveal relationships between ubiquitylation and protein fate. We sought to determine whether quantitative proteomics could be used to identify proteins for which changes in ubiquitylation alter protein abundance in a signal-dependent manner. To this end, we utilized stable isotope labeling of amino acids in cell culture (SILAC) in combination with immunoprecipitation of "ubiquitin remnant" peptides (peptides with modified lysine residues) and mass spectrometry to quantify changes in protein-specific ubiquitylation in CD4+ T cells stimulated via the TCR compared to resting T cells. SILAC quantification revealed dynamic changes in ubiquitylation of hundred of proteins, including key signaling transducers, during TCR stimulation. To determine whether these ubiquitylation events predict robust post-translational control of protein abundance, we performed whole cell proteomic analysis to quantify changes in relative protein abundance during T cell activation. Comparing changes in ubiquitylation with changes in protein abundance in stimulated CD4+ T cells, we identified a subset of proteins for which ubiquitylation is tightly linked to protein abundance. Efforts are now underway to test the hypothesis that changes in ubiquitylation during T cell activation alters the abundance of specific T cell effector proteins and signaling intermediates, and thus finely tunes the T cell response.

P24. "CyTOF, a new generation mass cytometer available in IFI, UPENN, and VA Medical Center"

Takuya Ohtani

The CyTOF2 is a new generation mass cytometer, which utilizes Time-Of-Flight mass spectrometer as a readout. The system uses heavy metal labeled antibodies instead of fluorescent–labeled antibodies, which allows multi-parametric single cell analysis for more than 40 channels with minimal background and compensation problems.

Here we would like to present representative data obtained by CyTOF. We are able to identify major peripheral blood subsets including Granulocytes, Basophils, Plasmacytoid Dendric Cells, NK cells, Myeloid Dendric Cells, Non-Canonical Monocytes, Canonical Monocytes, Platelets, B cells, Helper T cells and Killer T cells, using 17 marker antibody panel. The phosphorylation of S6kinase, ERK1/2, and p38 in response to PMA/ionomycin stimulation is clearly identified in these populations as well. We have also successfully developed protocols for bar-coding, which allows unbiased and precise analysis together with a higher throughput.

IFI 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.

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P25. "Caspase-8 activity regulates cell-intrinsic TLR-induced gene expression independently of cell death"

Naomi Philip

Toll-like receptors (TLRs) sense conserved microbial structures such as lipopolysaccharide (LPS) or peptidoglycan, and activate signaling pathways that induce anti-microbial gene expression programs that include both inflammatory mediators and cell survival proteins. Interestingly, when these pro-survival signals are dampened during microbial exposure, TLR signaling can elicit cell death via caspase-8-mediated apoptosis. Caspase-8 functions as a pro-apoptotic factor, but paradoxically can also promote cell survival by blocking an alternative form of cell death known as programmed necrosis, regulated by the receptor interacting protein kinase 3 (RIPK3). These disparate functions are regulated by distinct caspase-8 binding partners. Specifically, caspase-8 homodimerization leads to autoprocessing and activation of apoptosis, whereas caspase-8 heterodimerization with the catalytically inactive homologue cFLIP inhibits both apoptosis and programmed necrosis. We now find that in addition to its

well-established role in regulating cell death pathways, caspase-8 plays an important role in the cell-intrinsic expression of inflammatory genes expression independent of cell death. In particular, we find that cells lacking caspase-8 and RIPK3, but not RIPK3 alone, have a defect in expression of a large subset of TLR-induced inflammatory cytokines. Unexpectedly, our data reveal that autoprocessing of caspase-8 is necessary for its ability to facilitate TLR-induced gene expression. Our findings provide new insight into the role of caspase-8 in host defense and demonstrate that caspase-8 enzymatic activity has a non-apoptotic function in control of inflammatory gene expression.

P26. "Rapid evolution of SIV-specific CD8 T cell cytolytic potential during acute SIV infection"

Emily Roberts

CD8+ T cells are critical for control of viremia during HIV infection. During chronic HIV infection, loss of CD8+ T cell cytotoxic activity, controlled in part by the transcription factor T-bet, is associated with disease progression. However, it is unclear whether CD8+ T cell dysfunction evolves progressively throughout infection, or is established during acute infection. To address this question, we infected MamuA*01+ rhesus macaques (RM, n=18) i.v. with SIVmac251 and examined the kinetics of SIV-specific CD8+ T cell evolution within the intestinal mucosa via sequential biopsy and necropsy, and in mesenteric lymph nodes (mLN) and spleen through 90 days post-infection. CD8+ T cells from various regions of the gut mucosa (rectum, ileum, jejunum, colon) were evaluated by flow cytometry for SIV-specificity (MamuA*01 gag-CM9 and tat-TL8 tetramers), T cell activation (CD38, Ki67), and cytolytic potential (GrzB, Perf, T-bet). Compared to total memory CD8+ T cells, SIV-specific CD8+ T cells were disproportionately activated in every region of the gut , mLN and spleen during acute SIV infection. SIV Tat-specific CD8+ T cells decreased both in frequency and activation status (ki67) after resolution of acute viremia, whereas SIV Gag-specific CD8+ T cell decreased in frequency in most infected animals but retained higher activation levels into the chronic phase in both gut and mLN. Cytolytic potential was acquired rapidly in both Gag and Tat-specific CD8+ T cells, but progressively lost after peak viremia. T-bet expression among SIV-specific CD8+ T cells within gut mucosa and mLN was minimal. These data indicate rapid evolution and loss of cytolytic function in mucosal and mLN SIV-specific CD8+ T cells after acute SIV infection, which may have qualitatively reduced cytolytic capacity.

P27. "Blockade of IL-33/ST2 signaling enables CD8+ T cell exhaustion and prolongs survival in the murine model of familial hemophagocytic lymphohistiocytosis"

Julia Rood

Cytokine storm syndromes, such as familial hemophagocytic lymphohistiocytosis (FHL), are lethal disorders caused by uncontrolled, systemic immune activation. In the murine model of FHL, perforin-deficient (Prf1-/-) mice infected with LCMV, disease is driven by overabundant IFNγ-producing LCMV-specific CD8+ T cells. We now show a novel role for the alarmin IL-33 in amplifying this immune dysregulation in FHL. Blockade of the IL-33 receptor, ST2, markedly improves survival of LCMV-infected Prf1-/- mice ($p<0.001$) and reduces the severity of multiple disease parameters, including serum IFNγ ($p<0.001$). This decrease in IFNγ corresponds to a reduction in both the frequency of IFNγ+ LCMV-specific CD8+ T cells ($p<0.001$) and the magnitude of IFNγ expression in these cells ($p<0.001$) eight days post-infection. Over time, ST2 blockade leads to phenotypic and functional exhaustion of LCMV-specific CD8+ T cells, as evidenced by upregulation of inhibitory receptors (PD-1, $p<0.001$; 2B4, $p<0.001$), decreased ratio of T-bet:Eomes expression ($p<0.001$), and reduced cytokine production (IFNγ, $p<0.001$) by 30 days post-infection. Disruption of IL-33 signaling is only required early in the course of infection to mediate CD8+ T cell exhaustion, as withdrawal of ST2 blockade 15 days post-infection fails to reverse this long-term loss of function. These findings demonstrate that ST2 blockade is protective in murine FHL by enabling exhaustion of otherwise immunopathologic cells. Furthermore, our results suggest that modulation of IL-33/ST2 signaling may be a useful strategy for tuning CD8+ T cell exhaustion in the context of autoimmune disease and/or chronic viral infection.

P28. "Factors Controlling the Adoption of Plasma Cell Fate"

Rebecca Rosenthal

Humeral immunity requires B-cell to plasma cell differentiation. However, B-Cells and plasma cells are separated by a mutually repressive transcriptional network with B-Cell factors Bcl6, Bach2, and Pax5 blocking the expression of plasma cell factors, Blimp1, Irf4, and Xbp1, which in turn inhibit the expression of B-Cell factors Bcl6 and Pax5. It is unclear how B-Cells overcome this initial repression to differentiate into plasma cells. Since plasma cell differentiation is linked to cell division, we sought to further define this relationship and test if down-regulation of transcription factors restricting plasma cell differentiation precedes plasma cell fate adoption. Follicular B-Cells were isolated from Blimp1gfp/+ reporter mice and labeled to track cell division and cultured under plasma cell inducing conditions for 3 days. Cells which had undergone various numbers of divisions but not become plasma cells along with GFP+ plasma cells were sorted and processed for RNA. rtPCR was performed to measure Bach2, Bcl6, Pax5, Irf4, and Blimp1 expression. While Bach2 and Pax5 expression levels remained relatively similar in Blimp1-negative B-Cells which had undergone different numbers of divisions, Bcl6 expression levels displayed a downward trend with increasing division number. Similarly, marginal zone B-Cells, which are known to become plasma cells with increased kinetics and decreased division requirements, expressed lower levels of Pax5, Bcl6, and Bach2. These results imply that Bcl6 gene expression levels may be important in initiating antibody secreting cell differentiation and that decreasing levels of Bcl6 from cell division may be important for plasma cell differentiation.

P29. "Regulation of the mosquito midgut bacteria by the steroid hormone 20-hydroxyecdysone"

Sarah Sneed

"Anopheles gambiae and Aedes aegypti are mosquito species that cause high morbidity and mortality by spreading pathogens such as Plasmodium parasites and arboviruses. As the midgut of the mosquito is the main barrier to dissemination of pathogens from a bloodmeal into the hemolymph, understanding midgut immune mechanisms and their regulation is critical. Blood feeding triggers signaling pathways required for successful egg development. We hypothesized that these pathways may play additional roles in modulating immunity. The steroid hormone 20-hydroxyecdysone (20E) signaling cascade plays an important role in egg development. 20E is rapidly produced in the ovaries in response to blood feeding and is released into the hemolymph where it can signal to peripheral organs. Previously, studies of the relationship between 20E and mosquito immunity have been focused on 20E's effect on the fat body. To date, no studies have examined the midgut as a potential target of 20E signaling. We found directly injecting 20E into non-blood fed Ae. aegypti and An. gambiae resulted in a significant increase in midgut bacteria measured by colony counting and 16S qPCR. The increase in bacteria parallels what is observed after blood feeding. Metagenomic, transcriptomic, and functional analyses of 20E treated mosquitoes are underway to explore mechanisms leading to expansion of midgut bacteria. Our work provides evidence that 20E signaling influences midgut bacteria levels and that this mechanism is conserved across diverse mosquito species. Understanding regulation of the mosquito commensal population is crucial as their abundance and species composition have been implicated in disease transmission.

P30. "Regulation of effective vs. ineffective B cell responses to chronic viral infection"

Ryan Staupe

HIV and HCV are chronic viral infections that cause significant morbidity and mortality in millions of people worldwide. Current vaccine efforts 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. Thus, the overarching-goal of this project is to interrogate the cellular and molecular events that lead to the delayed and/or altered production of effective antiviral antibodies during chronic viral infection. We have developed a technique that allows the track the virus-specific B cell response in the LCMV model, a mouse model of chronic infection. Using this technique, we have found that chronic viral infection causes a skewing of the virus-specific B cell response to the plasma cell fate and away from the germinal center fate. These results suggest that altered 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 viral infection.

P31. "Defining the role of the miR-181 family in visceral adipose tissue inflammation, insulin resistance, and obesity"

Anthony Virtue

The global incidence of overweight and obese individuals has risen to 1.7 billion people worldwide, resulting in a parallel outgrowth in chronic inflammatory metabolic disorders such as type 2 diabetes and cardiovascular disease. In light of this growing health crisis, we have concentrated our efforts in evaluating the underlying mechanisms that drive the development of insulin resistance, a hallmark in the pathogenesis of obesity-associated diseases. Over the past decade, it has become clear that microRNAs are key regulators of immune cell function and cellular metabolism; however, very little is known on how microRNAs impact the chronic inflammatory processes that occur within visceral adipose tissue during obesity. Specifically, we have focused on the miR-181 family since its members are highly expressed in hematopoietic cells and are detectable in metabolically relevant tissue. With the use of murine models, we have made several important discoveries: first, all miR-181 family members are significantly upregulated in visceral adipose tissue during diet-induced obesity; second, global genetic deletion of 4 out of 6 miR-181 family members leads to significantly reduced total fat mass and enhanced glucose tolerance; and finally, evaluation of the prominent adipose tissue cell types such as CD4 T-cells, Tregs, macrophages, and adipocytes led us to identify the specific cell type accountable for the enhanced glucose tolerance phenotype. In conclusion, we have determined that the expression of miR-181 family members is augmented in diet-induced obesity and that their cell specific expression can result in altered glucose tolerance.

P32. "Enhanced Myelopoiesis Downstream of Toll-Like Receptor 9 Activation Drives a Feed-Forward Inflammatory Response"

Lehn Weaver

Monocytes are innate immune cells that interact with their environment through the expression of pattern recognition receptors, including Toll-like receptors (TLRs), which are activated by endogenous and exogenous ‘danger’ signals. These signals are critically important for the maintenance of innate protection against pathogen challenge, but have also been implicated in driving persistent inflammation in sterile inflammatory conditions, such as atherosclerosis and autoimmunity. Although efforts to understand monocyte biology have demonstrated key regulatory mechanisms controlling bone marrow monocytopoiesis during homeostasis, less is known about the regulation of monocytopoiesis during systemic inflammatory responses. Herein, we leverage our understanding of a model systemic inflammatory disease to demonstrate new insight into inflammation-induced monocytopoiesis. Firstly, we demonstrate that repeated stimulation through TLR9 leads to a feed-forward inflammatory response correlated with increased production of inflammatory monocytes from an expansion of extramedullary myeloid progenitors. These extramedullary myeloid progenitors not only accumulate in peripheral tissues, but are also reprogrammed to have increased inflammatory monocyte production capacity. Intriguingly, CCR2-/- mice are not protected from TLR9-driven inflammation, as extramedullary myelopoiesis sustains in situ production of monocytes and correlates with tissue-specific levels of inflammation. This is the first demonstration that peripheral monocytosis can develop in the absence of CCR2, and demonstrates the capacity of in situ monocytopoiesis in peripheral tissues to contribute to systemic inflammation. These findings expand our understanding of how monocytes are produced and positioned during systemic inflammatory responses providing new insight into monocyte biology with broad implications for the treatment of systemic inflammatory diseases.

P33. "Interference by Circulating Antibodies in De Novo Antibody Responses to Influenza"

Elinor Willis

Neonates of many species are highly susceptible to infection and severe disease from a variety of pathogens. Maternal antibodies are an important part of the neonatal immune system. Both mammalian and avian females transfer protective antibodies to neonates that are generated against pathogens in their immediate environment during or after gestation. However, maternal antibodies can also hinder the neonate's own antibody responses, such as to vaccination. The mechanism by which this hindrance occurs is not clear. We established a mouse model to study the role of maternal antibodies in the context of influenza infection. We are also using a passive transfer model to shed light on the mechanism by which circulating antibodies can interfere with the generation of de novo antibody responses. Preliminary results indicate that maternal antibodies reduce the magnitude of the de novo antibody response against influenza virus. Despite this, and in contrast to adult mice, neonatal mice go on to develop long-lived immunity following influenza exposure. Ongoing experiments are addressing if these long-lived immune responses are T cell-mediated or antibody-mediated. We will also investigate mechanisms underlying the observed difference in adult vs. neonatal immunity in the presence of circulating antibodies.

P34. "Inflammasome activation is required for the generation of protective immunity against Salmonella"

Meghan Wynosky-Dolfi

Salmonella enterica species cause disease ranging from severe gastroenteritis to typhoid. Widespread antibiotic resistance limits our ability to inhibit these pathogenic infections. Defining factors that drive protective immunity against Salmonella may help to develop therapeutics for controlling infections. Induction of anti-bacterial immune defense requires coordination of multiple arms of innate and adaptive responses. NOD-Like Receptors (NLRs) are cytosolic proteins that activate the inflammatory caspases, caspase-1 and -11 to induce pro-inflammatory cell death and release of IL-1 family cytokines in response to pathogen virulence. Release of intracellular components from dying cells and IL-1 cytokines promote the recruitment and activation of monocytes and neutrophils. This response facilitates generation of pathogen-specific T- and B-cell responses that aid in clearance of infection and generate pathogen-specific memory responses. The role of inflammasome activation in induction of protective immune responses against mucosal bacterial pathogens is not well defined. To examine the role of inflammasome activation in generating protective immune responses, we orally vaccinated B6 and Casp1-/-Casp11-/- mice against Salmonella, then orally challenged mice with a lethal dose of Salmonella. Interestingly, Casp1-/-Casp11-/- mice had a severe defect in generating protective immunity. Notably, these mice were unable to generate Salmonella-specific mucosal and systemic antibodies, and had a significant defect in priming of pathogen-specific CD4+ T cell responses. Il1r-/- mice had no defect in Salmonella-specific antibody production and were resistant to lethal challenge following immunization. These data suggest that inflammasome activation plays a crucial role in the generation of protective pathogen-specific mucosal immune responses independent of IL-1 signaling.

P35. "Granzyme B mediates regulation of immunopathology in leishmaniasis"

Scarlett Yang

Leishmaniasis is a disease that affects millions of individuals worldwide and results in a wide array of clinical presentations. Although immune responses against this parasitic infection, such as the type 1 response, can be beneficial, excessive immune activation leads to the pathology observed in patients. Thus, defining the mechanisms that regulate immune responses and limit immunopathology is pivotal to disease control. Regulatory T cells (Treg cells) have been reported to limit inflammation in a granzyme B (GzmB)-dependent manner. Here, we demonstrate that Treg cells upregulate GzmB during Leishmania major infection. The Treg cells that express the transcription factor T-bet are enriched in the GzmB+ subset, and T-bet expression correlates with higher levels of degranulation by Treg cells. Furthermore, GzmB deficiency leads to exacerbated pathology in mice after infection, while parasite burden does not increase. Taken together, these data suggest that GzmB mediates suppression of immune responses and may be a crucial mechanism by which Treg cells function during Leishmania infection.

P36. "Regulation of Immunity to Neuroblastoma Via Polyamine Blockade"

Adriana Benavides

Neuroblastoma (NB) accounts for a considerable portion of childhood cancer-related mortalities. Despite improvements in therapy, survival rates in high-risk patients remain poor. High-risk disease results from MYCN-amplification and alterations in Myc-regulated pathways such as polyamine (PA) synthesis. MYCN-amplified, high-risk NBs have elevated PA levels due to Myc's control of ornithine decarboxylase, the rate-limiting enzyme for PA synthesis. In a mouse MYCN-driven NB model, PA blockade using difluoromethylornithine (DFMO) led to greater reduction in NB burden than that anticipated by DFMO's in-vitro capacity to control tumor growth, suggesting that PA blockade may result in tumor-cell extrinsic effects. Indeed, elevated PAs can promote the differentiation and function of tumor-supportive cells, while PA blockade can reverse immune-suppression by increasing tumor-infiltrating leukocytes (TILs). However, previous studies investigating the effects of PA blockade involved in-vitro analysis using cell lines or in-vivo analysis using immune-deficient mouse models or non-spontaneous tumor models. Therefore, we sought to characterize the NB microenvironment in immune-competent TH-MYCN+/+ transgenic mice with and without DFMO. Tumors at terminal disease were mechanically and enzymatically dissociated, and the frequencies of various TILs were assessed using an optimized flow cytometry-based protocol. Our results indicate that DFMO reproducibly alters the cellular composition of the NB microenvironment. We found an increased frequency of dendritic cells, natural killer cells, and a maintenance of CD4-negative invariant natural killer T-cells and tumor-associated, granulocytic myeloid-derived suppressor cells. These data support our hypothesis that PA blockade induces distinct tumor-cell extrinsic changes in the microenvironment that allow for more efficient immune control of NB.

P37. "Identification of a Natural Viral RNA Motif that Optimizes Sensing of Viral RNA by RIG-I"

Jie Xu

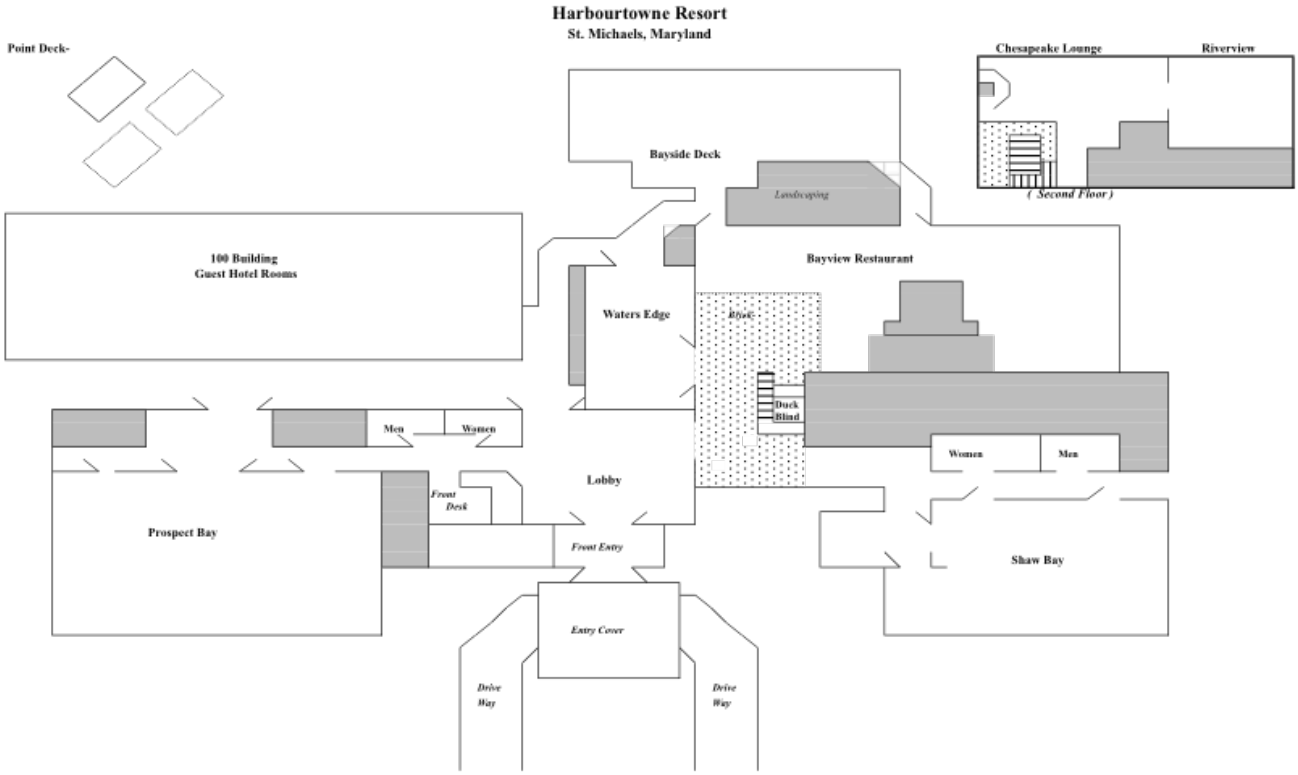
Stimulation of the antiviral response depends on the sensing of viral pathogen-associated molecular patterns by specialized cellular proteins. During infection with RNA viruses, 5'di- or triphosphates accompanying specific single or double-stranded RNA motifs trigger signaling of intracellular RIG-I-like receptors (RLRs) and initiate the antiviral response. Although these molecular signatures are present during the replication of many viruses, it is unknown whether they are sufficient for strong activation of RLRs during infection. Immunostimulatory defective viral genomes (iDVGs) from Sendai virus (SeV) are among the most potent natural viral triggers of antiviral immunity. Here we describe an RNA motif (DVG70-114) that is essential for the potent immunostimulatory activity of 5' triphosphates-containing SeV iDVGs. DVG70-114 enhances viral sensing by the host cell independently of the long stretches of complementary RNA flanking the iDVGs and it retains its stimulatory potential when transferred to otherwise inert viral RNA. In vitro analysis showed that DVG70-114 augments the binding of RIG-I to viral RNA and promotes enhanced RIG-I polymerization, thereby facilitating the onset of the antiviral response. Together, our results define a new natural viral PAMP enhancer motif that promotes viral recognition by RLRs and confers potent immunostimulatory activity to viral RNA.

P38. "Using chimeric BET proteins to study how they bind chromatin and contribute to transcription"

Michael Werner

Bromodomain and extra-terminal motif (BET) proteins BRD2, BRD3, and BRD4 bind chromatin and "read" acetylated lysines to cue transcription. Inhibition of the acetyl-binding BET bromodomains has proven effective in treating inflammation, cardiac remodeling, and many cancers. Despite excitement in this new field of therapy, a comprehensive understanding of how BET proteins bind to a given locus and contribute to transcription is lacking. Using a gain-of-function approach in a BRD2-/- cell line, we observed that BRD3 but not the short or long isoforms of BRD4 (BRD4s and BRD4l) can rescue transcription, suggesting that functional overlap exists between BRD2 and BRD3 but not BRD4. Given that these two proteins are structurally similar to BRD4s, we designed chimeric BRD2 and BRD4s constructs to determine in an unbiased manner which domains underlie BRD2 function. Intriguingly, a chimera containing the bromodomains of BRD4 and the carboxy-terminal domains of BRD2 was sufficient to rescue BRD2-/- cells. Future experiments will refine our domain mapping of BET proteins to understand how individual domains contribute to localization on the genome, recruitment of transcriptional cofactors, and subsequent transcriptional control.

Meeting Rooms



Resort Grounds

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