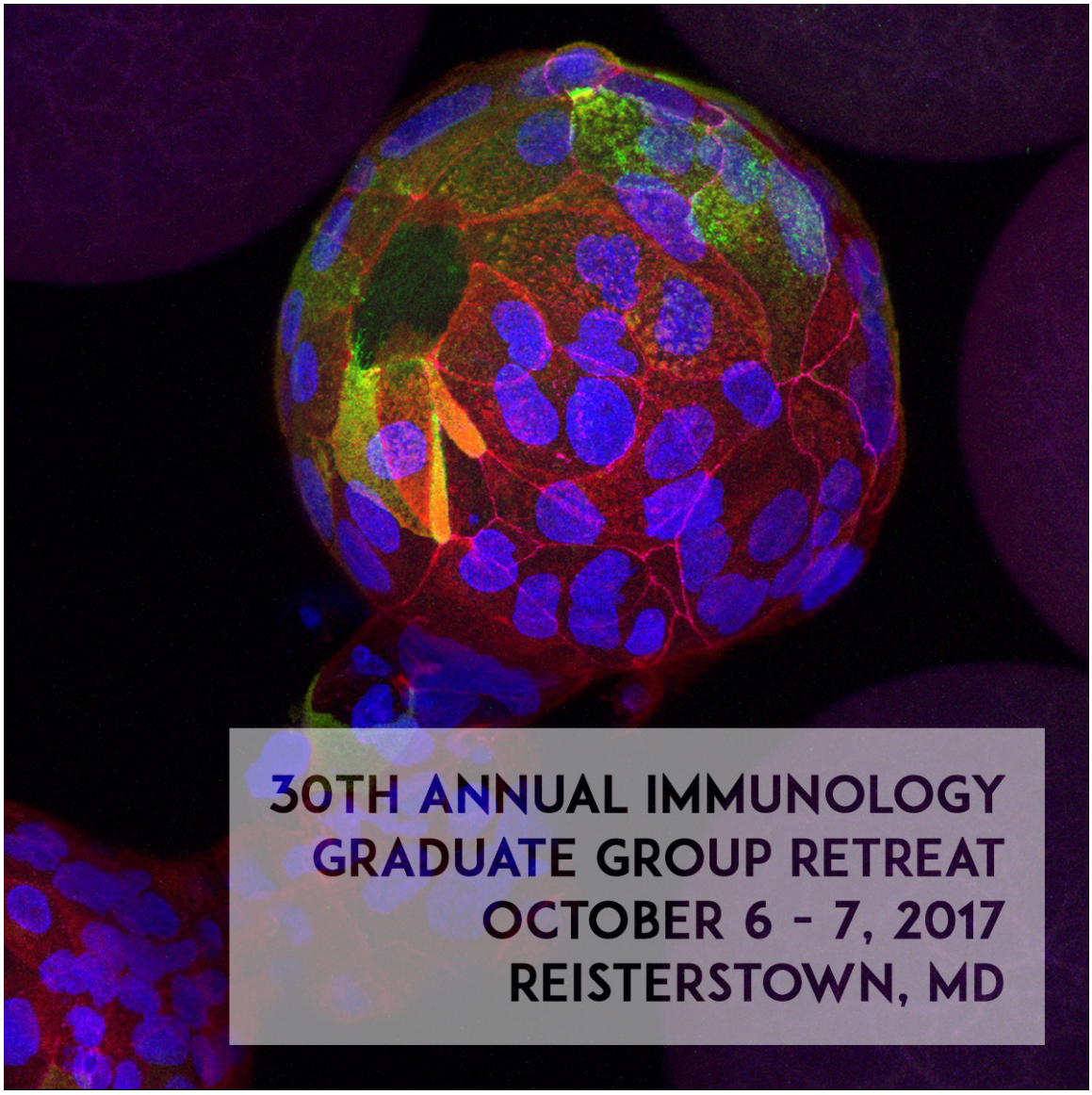

30TH ANNUAL IMMUNOLOGY GRADUATE GROUP RETREAT

OCTOBER 6 - 7, 2017

REISTERSTOWN, MD

FRIDAY, OCTOBER 6

11:00 AM – 1:30 PM	**Registration** (Lobby)
12:00 – 1:30 PM	**Lunch** (Harvest Dining Room)
1:30 – 1:40 PM	**Welcome** (Jubilee) Bruce Freedman, VMD, PhD and Michael May, PhD
1:40 – 3:30 PM	**Session I: Genetic Regulation of Immunity** (Jubilee) S1A. Brian Gaudette, PhD "B cell-intrinsic epigenetic priming of plasma cell differentiation" S1B. Glendon Wu "Investigating the roles of recombination signal sequences in promoting mono-allelic *Tcrb* assembly and preserving genomic integrity" S1C. Thomas Burn "Expanding the Dogma: Unconventional translation of alternate RAG1 isoforms and implications for T cell development, selection, and autoimmunity" S1D. John Johnson (session chair) "Critical role of Tcf-1 in shaping the chromatin accessibility in naïve T cells" S1E. Ling Zhao, MD, PhD, Technical Director, Human Immunology Core "An Introduction to the Assays and Services provided by the Human Immunology Core facility at Penn"
4:00 – 5:45 PM	**Session II: Control of Immune Cell Function and Development** (Jubilee) S2A. Ethan Mack "Trib1 regulates eosinophil identity by restraining the neutrophil identity program" S2B. Elisabet Bjanes "A novel role for CARD19 in control of cell death and anti-bacterial host defense" S2C. Corbett Berry "Calcium dependent mechanisms control T lymphocyte exit from quiescence" S2D. Omar Khan (session chair) "The HMG-Box Protein Tox Induces T Cell Exhaustion to Maintain Durable Responses to Chronic Infection" S2E. Enrico Radaelli, DVM, PhD, Technical Director, Comparative Pathology Core "The Comparative Pathology Core (CPC): the value of animal pathology in biomedical research"
5:45 – 7:00 PM	**Dinner** (Harvest Dining Room)
7:00 – 8:00 PM	**New Faculty Talk: Boris Striepen, PhD** (Jubilee) Professor, Pathobiology, Penn School of Veterinary Medicine "Cryptosporidium: a new model to interrogate intestinal infection"
8:00 PM – 12:00 AM	**Reception and Campfire** (Lobby, upstairs and downstairs)

SATURDAY, OCTOBER 7

8:00 – 9:00 AM	**Breakfast** (Harvest Dining Room)
9:00 – 10:40 AM	**Session III: Regulation of the Immune Response** S3A. Emily Moser, PhD "Germinal center B cells are regulated by the ubiquitin ligase Itch" S3B. Elinor Willis "A genetic-based vaccine overcomes maternal antibody inhibition of immune responses" S3C. Oliver Harrison, DPhil "Local tuning of commensal-specific immunity" S3D. Walter Mowel "Group 1 innate lymphoid cell lineage identity is determined by a cis-regulatory element marked by a long non-coding RNA" S3E. Jeffrey Lin (session chair) "CD40 Agonist Partially Rescues Systemic Dysregulation of CD103+ Type 1 Classical Dendritic Cells in KPC Tumor-Bearing Mice"
11:00 AM – 12:15 PM	**Keynote: Kristin Hogquist, PhD** (Jubilee) Professor, Center for Immunology, Department of Laboratory Medicine and Pathology, University of Minnesota "Thymic selection of soldiers and peacekeepers"
12:15 – 12:45 PM	**Group Photo**
12:45 – 2:00 PM	**Lunch** (Harvest Dining Room)
2:00 – 3:00 PM	**Career Development for Trainees: Grants 101** (Orchard) Leslie King, PhD, Grants Editor, Penn School of Veterinary Medicine
3:00 – 5:00 PM	**Free Time**
5:00 – 6:15 PM	**Dinner** (Harvest Dining Room)
6:15 – 8:00 PM	**Poster Session and Awards** (Lobby, Orchard)
8:00 PM – 12:00 AM	**Reception featuring NIH's Affordable Rock and Roll Act (ARRA)** (Orchard)

SUNDAY, OCTOBER 8

8:00 – 9:30 AM	**Breakfast** (Harvest Dining Room)
10:00 AM	**Checkout Deadline**

***Cover Photo** // Three-dimensional 'organotypic' cultures of intestinal epithelium (Caco-2 cells) generated in a Rotating Wall Vessel Bioreactor. In this environment, cells polarize and differentiate to form specialized secretory cell types surrounding collagen-coated beads. Nuclei are stained with DAPI (blue) and Actin with phalloidin (red). Mucins and mucous-producing cells are labeled with the lectin, UEA-1 (green).*

Image captured in the PennVet Imaging Core courtesy of Elaine de Martinis and Daniel Beiting.

ABSTRACTS
Talks & Posters

S1A. "B cell-intrinsic epigenetic priming of plasma cell differentiation"

Brian Gaudette, PhD, David Allman

The generation of antibody secreting plasma cells (PCs) from mature naïve B cells is thought to be a tightly regulated process closely linked to cell division. Indeed, follicular (Fo) B cells upon activation undergo successive rounds of division before acquiring the necessary shift in transcriptional regulators required to begin the PC program. However, unlike naïve Fo B cells, marginal zone (MZ) B cells are able to rapidly differentiate to PCs with less stimulation. In this study we developed a system in which the activating and PC-inductive programs can be uncoupled in Fo B cells and not in MZ B cells. Using this system we show that in resting MZ B cells the PC-inductive program is primed for rapid progression to the PC effector program in a mitosis-independent manner. Our results elucidate an epigenetic and transcriptional landscape in MZ B cells with striking similarity to that of PCs, which includes PC-relevant protein expression and increased mTORC1 signaling yet exists in the absence of extrinsic activation. Finally, we show that Fo B cells acquire these PC-priming characteristics seen in MZ B cells only after activation and successive rounds of cell division.

S1B. "Investigating the roles of recombination signal sequences in promoting mono-allelic ***Tcrb*** assembly and preserving genomic integrity"

Glendon Wu, Katherine Yang-Iott, Craig Bassing

The loci harboring antigen receptor (AgR) genes contain arrays of variable (V), diversity (D), and joining (J) gene segments flanked by recombination signal sequences (RSSs). The RAG endonuclease binds and cleaves at RSSs to assemble complete AgR genes via the process of V(D)J recombination. V(D)J recombination of ***Tcrb***, ***Igh***, and ***Igk*** loci is regulated such that AgR genes are assembled on and expressed from only one allele (allelic exclusion) in most cells. It is assumed that allelic exclusion is achieved by mono-allelic initiation and feedback inhibition of the V-to-(D)J recombination step, while intrinsic features of RSSs have been proposed to mediate mono-allelic V-to-DJ recombination of ***Tcrb*** and Igh loci. To investigate roles of RSSs in enforcing allelic exclusion via governing mono-allelic V-to-DJ recombination, we established and analyzed mice with the ***Trbv2*** or Trbv31 RSS replaced by an RSS that possesses greater intrinsic recombination activity. Each of these RSS substitutions causes a profound increase in the development of T cells expressing either Trbv2+ or Trbv31+ TCRβ, reflecting that RSSs flanking ***Trbv*** segments are major determinants of ***Trbv*** recombination frequency. Strikingly, mice with Trbv2 and Trbv31 RSS replacements on opposite alleles exhibit a 30-fold increase in cells expressing both Trbv2+ and Trbv31+ TCRβ chains (allelic inclusion). Our data suggests that the infrequency of ***Trbv*** recombination, as determined by inherent inefficiencies of ***Trbv*** RSSs, limits bi-allelic assembly of ***Tcrb*** genes within the time window before feedback inhibition permanently cements allelic exclusion. To investigate this possibility, we are pursuing experiments to confirm that these RSS substitutions increase the frequency of bi-allelic ***Trbv*** recombination without affecting chromatin accessibility, topology, or RAG binding of the ***Tcrb*** locus. We also are using these RSS replacement mice to test our hypothesis that the mechanisms directing mono-allelic V-to-(D)J recombination evolved from pressure to safeguard developing lymphocytes from oncogenic translocations.

S1C. "Expanding the Dogma: Unconventional translation of alternate RAG1 isoforms and implications for T cell development, selection, and autoimmunity"

Thomas Burn, Rahul Arya, Glendon Wu, Craig Bassing, Edward Behrens

The central dogma of molecular biology allows for great diversity within the proteome; however, it has become apparent that we may be severely underestimating the extent of this diversity. Protein translation in mammalian cells was thought to exclusively begin at the most 5' AUG start codon, flanked by a sufficiently strong Kozak sequence. Recently, transcriptome and proteome-wide studies have shown that this may not be absolute. RAG1 is an essential protein in the development of both T and B cell receptors. We have shown that RAG1 is translated from internal translation initiation sites, leading to N-terminally truncated isoforms. Pediatric patients

with frameshift mutations in the N-terminal region present with Ommen's syndrome and display concurrent autoimmunity and immune deficiency suggesting a break in either central or peripheral tolerance. Because these patients likely express RAG1 lacking N-terminal domains, it is possible that these regions of Rag1 are involved in maintaining proper selection of developing thymocytes. We have generated a mouse that mimics the mutations observed in some Ommen's syndrome patients and can only express the N-terminally truncated isoforms of RAG1. Using this mouse we show in a super-antigen model of negative selection that there is a defect in deletion of autoreactive thymocytes. Future studies will determine the mechanisms by which RAG1 N-terminal domains may be involved in preventing the escape of autoreactive T cells.

S1D. "Critical role of Tcf-1 in shaping the chromatin accessibility in naïve T cells"

John Johnson (session chair), George Georgakilas, Jelena Petrovic, Makoto Kurachi, Christelle Harly, Warren Pear, Avinash Bhandoola, E. John Wherry, Golnaz Vahedi

The symphonic interactions of transcription factors and the chromatin conducts cell-fate decisions through a multi-tiered regulatory procession. Although much is known about transcription factors required for various stages of T cell development, the transcription factors that effectuate the opening of chromatin—a fundamental layer of transcriptional activation—are unknown for T cells. To identify this unknown factor, we clustered genome-wide chromatin accessibility measurements across T cell development and uncovered sets of T-cell specific accessible regions. Motif analysis intimated a TCF transcription factor responsible for this ***de novo*** accessibility. Widespread binding and requirement of Tcf-1 for this T cell-specific accessible chromatin revealed the critical role of Tcf-1 in shaping the T cell chromatin landscape. In fibroblasts, ectopic expression of Tcf-1 orchestrated the gain of T cell chromatin accessibility and induced expression of T cell restricted genes—even at heterochromatic regions. Our results reveal an unprecedented means through which Tcf-1 controls T cell fate.

S1E. "An Introduction to the Assays and Services provided by the Human Immunology Core facility at Penn"

Ling Zhao, MD, PhD, Technical Director, Human Immunology Core

The Human Immunology Core (HIC) provides wet bench expertise and infrastructure support for early phase clinical trials and other investigations. HIC staff members perform blood and tissue processing; sample storage and shipment and a wide range of immunological assays including cytokine measurement by ***Digital ELISA*** and Luminex. HIC staff members also perform lymphocyte and innate immune cell subset analysis by ***flow cytometry*** and molecular immunology assays, including high throughput ***sequencing of immunoglobulin and T cell receptor*** gene rearrangements ***with single immune cell assays in development***. The HIC also offers ***purified immune cells*** from human apheresis donors for use by Penn investigators. The HIC designs and performs pilot experiments and generates standard operating procedures for clients. Finally, the HIC also offers investigators scientific expertise and guidance in immunology, assay design and validation, data analysis, technical and budget information for grant applications and regulatory compliance. You can learn more by searching "HIC" on the Penn website: ***https://pathbio.med.upenn.edu/hic/site/***

S2A. "Trib1 regulates eosinophil identity by restraining the neutrophil identity program"

Ethan Mack, Sarah J. Stein, Kelly S. Rome, Gerald B. Wertheim, Lanwei Xu, Warren S. Pear

Granulocytes, including neutrophils and eosinophils, are critically important for host defense; yet, there are significant gaps in understanding how granulocytes differentiate from bone marrow multipotent progenitors into mature effectors. The pseudokinase tribbles homologue 1 (Trib1) is an important regulator of granulocytes as knockout mice lack eosinophils and increase neutrophils through a C/EBPα-dependent mechanism. However, how and when Trib1 functions in this process is poorly understood. We evaluated the influence of Trib1 on eosinophil lineage commitment and identity. Stage-specific conditional Trib1 deletion revealed that Trib1 regulates eosinophil development at two points. Firstly, Trib1 supports eosinophil lineage commitment at the GMP to EoP transition. Secondly, Trib1 represses the neutrophil gene program during terminal eosinophil maturation. Our data provide critical insight into how precise transcription factor levels are required to balance mature granulocyte identity and illustrate a previously unknown plasticity during granulocyte development.

S2B. "A novel role for CARD19 in control of cell death and anti-bacterial host defense"

Elisabet Bjanes, Alexandra Delaney, Baofeng Hu, Brian C. Schaefer, Igor E. Brodsky

Regulated cell death is a conserved immune mechanism that contributes to the clearance of bacterial pathogens. Apoptosis and pyroptosis are caspase-dependent forms of regulated cell death induced by enteric bacterial pathogens, including ***Yersinia pseudotuberculosis*** and ***Salmonella*** Typhimurium, respectively. However, the mechanism by which the innate immune system determines cell fate following infection remains poorly understood. Apoptosis and pyroptosis are regulated by a family of related proteins that contain homotypic protein-protein interaction domains of the caspase recruitment (CARD) and death domain (DD) families. Here, we describe a new regulator of caspase-dependent death in bone marrow-derived macrophages (BMDMs) known as CARD19, or Bcl10-interacting protein with CARD (BinCARD). We demonstrate that CARD19-deficient BMDMs have substantial defects in caspase-dependent cell death in response to multiple stimuli including infection by ***Salmonella*** Typhimurium, ***Yersinia pseudotuberculosis***, and staurosporine. We also show that CARD19 localizes to the mitochondria, suggesting a potential role in mitochondrial stability or regulation of membrane potential. CARD19 colocalizes with another mitochondrial CARD-containing protein, MAVS (mitochondrial antiviral signaling protein), which forms a signaling scaffold that initiates interferon responses to viral pathogens. Unlike MAVS, however, CARD19 is not required for anti-viral responses. CARD19-deficient mice display significantly decreased survival and increased bacterial burdens when infected with ***Yersinia pseudotuberculosis***, indicating that CARD19 promotes host immune responses to bacterial infection. Future studies will dissect the mechanism by which CARD19 contributes to cell death and innate host defense against microbial infection.

S2C. "Calcium dependent mechanisms control T lymphocyte exit from quiescence"

Corbett Berry, Xiaohong Liu, Uri Hershberg, Christopher Lengner, Bruce Freedman

Antigen binding to the T cell antigen receptor (TCR) initiates IP3 mediated calcium (Ca^{2+}) release from the endoplasmic reticulum (ER). Sustained Ca^{2+} entry is initiated following Ca^{2+} release-dependent relocalization of the ER membrane resident STIM proteins and subsequent interaction with and activation of plasma membrane CRAC/Orai channels. The magnitude and pattern of Orai mediated changes in cytoplasmic Ca^{2+} concentration following TCR engagement reflect, in part, the choreography of antigen presentation and costimulatory influences provided by APCs. Importantly, sustained and/or high input Ca^{2+} signals are required for the induction of T cell proliferation and acquisition of effector functions; although we still do not have a full understanding of how Ca^{2+} coordinates T cell exit from quiescence and cell cycle entry. Using an unbiased transcriptional approach, we establish that loss of STIM/Orai dependent Ca^{2+} entry results in a profound loss of ***Myc*** transcription and Myc dependent gene expression. Furthermore, our studies demonstrate that TCR-induced Myc induction reflects 1) Ca^{2+} mediated NF-κB and NFAT dependent ***Myc*** transcription and 2) Ca^{2+} mediated mTORC1 dependent ***Myc*** translation. Ongoing efforts are focused on how Ca^{2+} signals generated by variations in antigen affinity and costimulation acting on these control points regulate T cell proliferation and the acquisition of effector functionality.

S2D. "The HMG-Box Protein Tox Induces T Cell Exhaustion to Maintain Durable Responses to Chronic Infection"

Omar Khan (session chair), John Wherry

Prolonged exposure to antigen, as often occurs in chronic infections and cancers, results in the functional "disarmament" of responding T cells. This process, termed T cell exhaustion, is characterized by the hierarchical suppression of critical effector functions, dampening of proliferative capacity, and loss of memory differentiation, ultimately limiting the ability of antigen-specific T cells to appropriately combat disease. Though numerous reports have implied important roles for transcription factors (TFs), inhibitory receptors (IRs), and soluble mediators in the development of T cell exhaustion, the molecular mechanisms that regulate the early events of this process remain poorly understood.

Utilizing computational approaches, we identified a highly conserved chromatin-associated protein, ***Tox***, that is strongly upregulated within 6 days of exposure to chronic infection but downregulated in effector and memory

T cells. Previous studies have classified Tox as a member of the high-mobility group (HMG) proteins. Though Tox has been shown to be critical for the development of NK, innate-like and CD4 T cells, its role in regulating peripheral T cell responses remains unexplored.

Here, we show that Tox maintains the pool of CD8 T cells responding to chronic infection by regulating a suite of cellular systems to dampen immune function and limit terminal effector differentiation. Tox expression is triggered by prolonged nuclear residence of NFAT2, resulting in the upregulation of multiple inhibitory receptors, reduced expression of TNFa and IFNg, and the re-expression of Tcf1. Loss of Tox in the setting of chronic infection results in the terminal differentiation and rapid depletion of responding T cells. Over-expression of Tox in T cells in vitro is sufficient to drive a significant proportion of the exhaustion gene signature and results in numerous epigenetic changes that mirror those found in vivo. These results suggest that Tox is critically required for the induction of exhaustion and the maintenance of a T cell pool that can continually respond to chronic infection.

S2E. "The Comparative Pathology Core (CPC): the value of animal pathology in biomedical research"

Enrico Radaelli, DVM, PhD, Technical Director, Comparative Pathology Core

Ly6C Characterization and validation of animal models of disease play a crucial role in our effort to understand and cure human disorders. Recognition and accurate interpretation of the clinicopathological endpoints in experimental animals are essential to achieving a better understanding of their preclinical relevance and translational potential.

As open resource platform, the Comparative Pathology Core (CPC) provides expert pathological characterization and validation of preclinical animal models and currently supports several major research lines within the University of Pennsylvania and affiliated institutes. Particular emphasis is given to the comparative pathology of genetically engineered mouse models in the context of both basic and translational research. To pursue this mission, the CPC offers the expertise of board-certified veterinary pathologists and has established a thorough comparative pathology pipeline for an accurate interpretation of the pathological changes developed in a wide range of experimental settings.

The CPC represents a unique resource embedded within the vibrant and internationally recognized research environment at the University of Pennsylvania and closely related institutes such as the Abramson Cancer Center, the Children's Hospital of Philadelphia and the Wistar Institute. The CPC is also conveniently connected with several state-of-the-art core facilities (including Penn Vet Imaging Core and Center for Host Microbial Interactions) which expertise can be particularly useful to complement the phenotypic characterization of the experimental animal models contributing to a better definition of their value.

S3A. "Germinal center B cells are regulated by the ubiquitin ligase Itch"

Emily Moser, PhD, Michael Cancro, Paula Oliver

Antibodies are powerful effectors of immune system function, and they can provide sterilizing immunity to pathogens as well as cause devastating tissue damage in many autoimmune diseases. Antibody secreting cells that differentiate from germinal center (GC) B cells are the major source of the long-lived antibody mediating both protective- and auto- immunity. However, the mechanisms within the GC that ultimately program the quality of antibody responses are not fully elucidated. The ubiquitin ligase ***Itch*** is a potent negative regulator of antibody responses; in the absence of Itch, both mice and humans develop systemic humoral autoimmunity, but the mechanism by which Itch limits antibody production is unknown. To begin to understand how Itch regulates antibody responses, we quantified splenic B cell populations in Itch KO mice, and we found that Itch KO mice display a marked elevation in activated B cells, that is GC B cells, memory B cells, and plasma cells, as well as increased serum IgG1 and IgM. To determine if Itch regulated activated B cells directly or indirectly, we generated mixed bone marrow chimeras with WT and Itch KO cells, and we found that Itch functioned in B cells to directly limit germinal center B cells. Additionally, after immunization with the model antigen NP-ova, Itch KO B cells maintained greater numbers of antigen specific GC B cells and plasma cells late in the response, and Itch KO plasma cells produced more high

affinity anti-NP antibody compared to WT B cells. To determine how Itch might limit accumulation of activated of antigen specific GC B cells and plasma cells late in the response, and Itch KO plasma cells produced more high affinity anti-NP antibody compared to WT B cells. To determine how Itch might limit accumulation of activated cells, we stimulated Itch KO and WT naïve B cells in vitro, and we found that Itch limited the extent of cell proliferation, but not survival or activation. Consistent with this, proteomics analysis from B cell lysate after activation revealed that Itch was a potent regulator of cell cycle, and identified several proteins that may be substrates of Itch ubiquitin ligase activity. Current studies are aimed at determining the molecular mechanisms by which Itch regulates cell cycle in activated B cells. Understanding how Itch controls antibody responses will identify new therapeutic targets for modulating humoral immunity and autoimmunity.

S3B. "A genetic-based vaccine overcomes maternal antibody inhibition of immune responses"

Elinor Willis, Norbert Pardi, Kaela Parkhouse, Drew Weissman, Scott E. Hensley

Infants are particularly vulnerable to infections and severe disease, including from influenza virus. One increasingly promising strategy to protect them during this period is through maternally derived immunity transferred to the neonate. Maternal antibodies (matAb) can protect the infant soon after transfer but wane over time, leaving the infant vulnerable again. Therefore, active immunity via vaccination must also be generated in the infant. Here, using a mouse model we show that influenza virus-specific matAb inhibit the development of infant antibody responses after infection with live influenza virus or vaccination with inactivated virus. However, a novel mRNA-based vaccine expressing an influenza virus protein was able to generate strong antibody responses in mice that possessed influenza virus-specific matAb, leading to long-lasting protection. Together, these results suggest that genetic vaccines can overcome matAb inhibition and elicit potent immune responses in infants.

S3C. "Local tuning of commensal-specific immunity"

Oliver Harrison, DPhil, Yasmine Belkaid

A dynamic dialogue between host and microbiota ensures that commensal colonization occurs as a state of mutualism, the breakdown of which is associated with chronic inflammatory disorders. We recently demonstrated that distinct commensal species drive unique cutaneous T cell responses, which are key to tissue homeostasis. We sought to understand how local tissue signals mould commensal-specific T cell function. Phenotypic and transcriptomic analysis of cutaneous T cells induced by commensals reveal that these cells are distinct from those responding to skin pathogens. Notably, amongst commensal-specific effector T cells, we observed a proclivity towards IL-17-producing CD8+ T cell differentiation, compared to a type-1 phenotype of pathogen-specific effector T cells. Strikingly however, commensal-induced IL-17 producing CD8+ T cells also demonstrated significant expression of Th2 gene transcripts, including Gata3, IL-5 and IL-13. As such, commensal-specific T cells maintain a poised/hybrid type-17/type-2 state of differentiation during steady state. Rapid production of type-2 cytokines by CD8+ T cells was elicited by local tissue alarmins, including IL-18. Furthermore, this poised/hybrid differentiation state was maintained by local Foxp3+ Treg cells, as attenuation of dermal Foxp3+ Treg cell function resulted in accumulation of committed, cytokine producing, Th2 and Tc2 cell populations following commensal colonization. Thus, homeostatic CD8+ T cell responses can rapidly respond to local cues to produce type-2 cytokines. Investigating the epigenetic and transcriptional events underlying generation of commensal-specific T cell responses during homeostatic immunity will aid our understanding of targets for treatment of chronic inflammatory disorders.

S3D. "Group 1 innate lymphoid cell lineage identity is determined by a cis-regulatory element marked by a long non-coding RNA"

Walter Mowel, Jorge Henao-Mejia

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

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

S3E. "CD40 Agonist Partially Rescues Systemic Dysregulation of CD103+ Type 1 Classical Dendritic Cells in KPC Tumor-Bearing Mice"

Jeffrey Lin (session chair), Robert H. Vonderheide

Pancreatic ductal adenocarcinoma (PDAC) now ranks as the third-leading cause of cancer-related deaths in the United States with an overall five-year survival rate of 9%. Recently, the treatment of select metastatic diseases like melanoma have been revolutionized by checkpoint blockade immunotherapy (CBI). CBI's block immune inhibitory factors expressed on tumor cells and stroma that dampen activity of tumor-infiltrating CD8+ T-cells. These dysfunctional CD8+ T-cells are subsequently reinvigorated, driving tumor eradication and durable clinical remissions in many patients. Unfortunately, CBI's remain ineffective in cancers such as PDAC in which CD8+ T-cell infiltration and cytolytic activity are relatively low. These features suggest a failure of dendritic cells to prime CD8+ T-cells against tumor-associated antigens in earlier stages of immune activation. How dendritic cells (DC's) are regulated in PDAC therefore demands investigation to determine whether their capacity to prime CD8+ T-cells is impaired and can be targeted therapeutically to broaden response to CBI's.

In the present study, migratory CD103+ type 1 classical DC's (cDC1's) are found to be suppressed systemically in the KPC mouse model of PDAC driven by Pdx1-Cre; $Kras^{G12D/+}$; $Trp53^{R172H/+}$. This cDC subset is essential for anti-tumor immunity due to their unique capacity for antigen cross-presentation and trafficking tumor antigen to draining lymph nodes. While all classical DC (cDC) subsets were found to be suppressed in the tumor itself and tumor-draining lymph nodes, CD103+ cDC1's were uniquely suppressed in distant lymph nodes systemically based on decreased expression of activation markers relative to healthy mice. Building upon prior work showing a critical

P1. "Adaptor protein-3 in dendritic cells limits autophagy to sustain inflammasome activation and control bacterial infection"

Adriana R. Mantegazza, Meghan A. Wynosky-Dolfi, Cierra N. Casson, Ariel J. Lefkovith, Sunny Shin, Igor E. Brodsky, Michael S. Marks

Inflammasomes assemble in the cytosol following phagocytosis of bacterial pathogens, but the molecular mechanisms linking phagosome dynamics to inflammasome regulation are poorly characterized. In dendritic cells (DCs), optimal Toll-like receptor signaling from phagosomes requires the endosomal adaptor protein-3 (AP-3). We show here that in murine DCs, AP-3 sustains inflammasome activation by phagocytosed stimuli by regulating inflammasome positioning and activation and by delaying inflammasome inactivation by autophagy. AP-3-deficient DCs hyposecrete IL-1β and IL-18 in response to phagocytosed stimuli in vitro, but IL-1β levels are restored by silencing autophagy. Concomitantly, AP-3-deficient mice exhibit higher mortality and produce less IL-1β and IL-18, and consequently less IL-17, than controls upon oral Salmonella typhimurium infection. Our data identify a novel link between phagocytosis, inflammasome activity and autophagy in DCs, potentially explaining impaired antibacterial immunity in AP-3-deficient patients and additional uncharacterized inflammatory disorders.

P2. "X-linked gene expression in splenic B-cells of a murine model of Systemic Lupus Erythematosus"

Anna Martin, Michael Atchison, Montserrat Anguera

Systemic lupus erythematosus (SLE) is a severe autoimmune disease that affects women at a rate nine times higher than men. The genetic basis for this bias is the X-chromosome, where the greatest concentration of immunity related genes on any chromosome can be found. Females have two X-chromosomes (XX), and through X-chromosome inactivation (XCI), silence one of their X-chromosomes randomly to have a similar level of X-linked gene expression as males (XY). Previous research has shown that human SLE patient B cells exhibit altered localization of XIST RNA, indicating that they have partial reactivation of the X_i. The hypothesis of this study is that due to improper Xist RNA localization, the expression of X-linked genes is increased in splenic B-cells of NZB/W F1 mice, which are a murine model of SLE. qPCR was performed using cDNA from splenic B-cells taken from female 3 and 7 month WT (n=1 each) and 3, 7, and 9 month NZB/W F1 mice (N=1, 2, and 2 respectively). Preliminary results indicate that the expression of TLR7 and CXCR3, two x-linked genes, are higher in splenic B cells of the 7 and 9 month old NZB/W F1 mice when compared to the 7 month old WT.

P3. "The loss of DGKζ protects from allergic asthma"

Brenal Singh, Taku Kambayashi

Asthma is a chronic allergic inflammatory airway disease that affects an estimated 300 million people worldwide. The pathogenesis associated with asthma is driven by Th2 CD4+ T cells, which upon TCR-dependent and TCR-independent activation release mediators that trigger airway inflammation and airway smooth muscle contraction. It has been shown that the differentiation of CD4+ T cells into Th1 and Th2 subsets may be dependent on TCR signal strength, whereby strong TCR-induced ERK signals favor Th1 over Th2 differentiation. We hypothesized that enhancement of TCR-mediated ERK signals would suppress the formation of an asthma-inducing immune response by promoting a Th1 response. To accomplish this, we targeted a negative regulator of diacylglycerol (DAG) signaling known as diacylglycerol kinase zeta (DGKζ) in order to selectively enhance DAG-mediated signals downstream of the TCR. Our preliminary data establishes a critical role for the regulation of DAG signaling in the development of asthma. DGKζ-deficient mice do not develop airway hyperresponsiveness (AHR) in an OVA-induced model of asthma. Furthermore, airway inflammation is also attenuated in DGKζ-deficient mice with decreased eosinophilia and Th2 cytokine levels (IL-4, IL-5, and IL-13) in the lungs of these mice. Unexpectedly, bone marrow chimera studies showed that the attenuation of airway hyperreponsiveness is due to the loss of DGKζ in the non-hematopoietic compartment while the reduction of airway inflammation is due to the

loss of DGKζ in the hematopoietic compartment. Furthermore, pharmacological inhibition of DGK using the pan-DGK inhibitor, R59949, was able to significantly reduce airway inflammation and AHR in OVA challenged mice, suggesting that acute loss of DGK function is sufficient to protect from OVA-induced asthma. Our studies demonstrate that the regulation of DAG controls the development of asthma and that inhibiting negative regulators of DAG can be a potential therapeutic strategy for the resolution of asthma by targeting both the immune and non-immune responses that drive the disease.

P4. "Enhancers regulate gene activation and pol2 pause release, but not pol2 binding rate, as revealed by correspondence between bursting and Pol2 ChIP"

Caroline Bartman, Cheryl A. Keller, Belinda M. Giardine, Ross C. Hardison, Gerd A. Blobel, Arjun Raj

Molecular biology studies provided a thorough understanding of how transcription works in terms of bulk biochemical behavior. However, transcription occurs in bursts, and it is unknown how transcriptional bursting relates to biochemical measures (e.g. Pol2 binding measured by ChIP). There is also disagreement on which step of transcription (gene activation, gene inactivation, polymerase initiation, or elongation) is the key regulatory step. We combined bursting measurements and Pol2 ChIP-seq using a quantitative framework, and found a very strong correlation between bursting parameters and Pol2 ChIP binding, allowing us to predict changes in bursting behavior genome-wide from Pol2 ChIP-seq data. Our quantitative model showed that Pol2 ChIP and bursting parameters would only correlate in this way if the rates of gene activation and Pol2 escape from pausing were key regulatory steps, whose rates are changed by stimuli. However, the rate of Pol2 binding was not limiting, and was not changed by perturbations, and neither was the gene closing rate. Our findings also allowed us to identify how different perturbations altered rates of gene activation and pol2 pausing escape genome- wide. Targeted mutagenesis of the mitoferrin enhancer revealed that all three hypersensitive sites regulate escape from pausing as well as gene activation rate. The combination of mathematical modeling with RNA FISH and Pol2 ChIP uncovered a fundamental connection between bursting and Pol2 binding patterns.

P5. "Protective Role of TLR9 Activation-Driven Heme Oxygenase 1 Expression-Dependent IL-10 Production by the Macrophages"

Chhanda Biswas, Ruth Choa, Taku Kambayashi, and Edward M Behrens

Heme degradation by heme oxygenase 1 (HO-1) and the metabolites including carbon monoxide (CO), ferrous iron, and bilirubin significantly contribute to cytoprotection, proliferation and anti-inflammation. Especially CO in IL-10-mediated anti-inflammatory responses is highly discussed. Earlier in our TLR9-macrophage activation syndrome (MAS) mouse model we demonstrated in an IL-10-limiting background the importance of proper IL-10 regulation in suppressing the pathology. However a role for TLR9 in HO-1-mediated immune-modulation is unknown. Here we demonstrate that the bone marrow-derived macrophages (BMDMs) in response to CpG induce both HO-1 and IL-10. This induction was 6-fold greater with respect to TLR4 activation. CORM2— a CO-releasing compound also responded similarly to CpG by producing a 3-fold higher IL-10. Simultaneously the levels of pro-inflammatory cytokines (IL-6, TNFα and IL-12) showed a reverse relation with IL-10 level. Precisely the inability of the HO-1- deficient BMDMs in CpG-driven IL-10 production suggests a direct role for TLR9-driven HO-1 expression-dependent IL-10 production. The HO-1 induction was TLR9 intrinsic because HO-1 level was not altered in TLR9-Knockout BMDMs and also by the production of secondary cytokines such as IL-6 or IL-10. Confirming further in vivo relevance in our TLR9-MAS mouse model, the repeat CpG injections significantly increased the expression of HO-1 in spleens. Future studies using HO-1 deficient mice will define the in vivo relevance of the HO-1 pathway for limiting systemic inflammation.

P6. "Reliance on lipid metabolism as a salvage pathway underlies functional differences of T cell subsets in poor nutrient environments"

Christopher Ecker, Lili Guo, Stefana Voicu, Luis Gil-de-Gómez, Andrew Medvec, Luis Cortina, Jackie Pajda, Melanie Andolina, Maria Torres-Castillo, Jennifer L. Donato, Sarya Mansour, Evan R. Zynda, Angel Varela-Rohena, Ian A. Blair, James L. Riley

T cells compete with malignant cells for limited nutrients within the solid tumor microenvironment. We found that effector memory CD4 T cells respond distinctly from other T cell subsets to limiting glucose and can maintain high levels of IFNγ production in a nutrient poor environment. Unlike naïve or central memory T cells, effector memory T cells fail to upregulate fatty acid synthesis and oxidative phosphorylation in limiting glucose. Interference of fatty acid synthesis in naïve T cells dramatically up-regulates IFNγ, while increasing exogenous lipids in media inhibits production of IFNγ by all subsets. These results demonstrate that T cells actively relying on fatty acid metabolism have impaired ability to produce IFNγ. Our studies clearly demonstrate a novel consequence for reliance on metabolic salvage pathways in nutrient poor conditions. Salvage pathways are not merely used for survival but can reshape cellular functionality. Together these data suggest that effector memory T cells are programmed to have limited ability to synthesize and metabolize fatty acids, which allows them to maintain T cell function in nutrient depleted microenvironments.

P7. "Modulation of T cell priming by dendritic cell stiffness"

Daniel Blumenthal, Vidhi Chandra and Janis K. Burkhardt

Priming of T-cell responses by dendritic cells (DCs) is essential for protective immunity to pathogen invasion and cancer. T cell activation requires intimate cell-cell interactions at a site termed the immunological synapse (IS) and mounting evidence indicates that this process involves mechanotransduction. In response to inflammatory stimuli, DCs undergo a maturation process during which their phenotype changes from one specialized for pathogen surveillance to one optimized for T cell priming. During maturation, T cell stimulatory ligands are upregulated, and cytoskeletal proteins are reprogrammed to downregulate antigen uptake and facilitate migration to lymphoid tissues. We hypothesized that maturation also alters the biophysical properties of the DC cortex, and that these properties represent an unexplored control point for T cell priming. Using atomic force microscopy, we show that upon LPS-induced maturation, DC stiffness increases from ~2kPa to ~4kPa in an actin-cytoskeleton dependent process. Using inhibitors and DCs from KO mice, we identify several actin regulatory pathways downstream of Rho GTPases involved in modulating DC stiffness. Interestingly, activating T-cells with agonist- coated acrylamide hydrogels of different compliance reveals a threshold for T cell activation within the range of 1-4 kPa, similar to the range over which the DC stiffness changes. The specific stiffness at which T cells are activated depends on the concentration of the stimulating molecules, indicating that mechanical cues are integrated with other stimulatory and co- stimulatory signals. Finally, by engineering DCs with altered stiffness, we show that the stiffness of mature DCs directly correlates with their ability to prime ex-vivo T-cells. Taken together, these findings indicate that the stiffness of the DC cortex provides a novel form of co-stimulation that has yet been considered.

P8. "Commensal microbiota contribute to interferon responsiveness in myeloid progenitors"

Danielle Minichino, Edward Behrens

During inflammation, hematopoiesis can be altered to produce more effector cells, such as myeloid cells, that perpetuate and amplify secondary immune responses. The mechanisms regulating hematopoiesis during inflammation remain elusive and understanding them are required for the development of novel therapeutics for uncontrolled inflammatory diseases. To date, we have demonstrated that depletion of intestinal microbiota using broad spectrum antibiotic treatment protects mice from inflammatory induced myelopoiesis in two different chronic inflammatory disease models. The current study investigates the cell intrinsic mechanisms in myeloid progenitors that are effected by antibiotic treatment. Myeloid precursors from an antibiotic treated mouse continue to have reduced myelopoietic capacity even when placed ***in vitro*** culture systems supplemented

with supportive cytokines. Genome–wide transcriptome analysis of myeloid progenitors highlights a hypo-responsiveness to interferon signaling. We then further confirmed this by testing interferon responses in vitro by determining interferon gamma induction of surface Sca-1. We show that precursor cells from antibiotic treated mice have impaired Sca-1 responses. Current research continues to delineate the exact mechanism by which the interferon gamma signal pathway is diminished in precursors from antibiotic treated mice, and its connection to the myelopoietic program.

P9. "Differential DNA damage responses among tissue resident macrophage populations"

Jacob Paiano, S. Tamoutounour, Y. Belkaid, A. Nussenzweig

Tissue resident (TR) macrophages are transcriptionally adapted to maintain their respective environments, from clearing infections to healing wounds. Many macrophage functions are tightly linked to changes in cellular metabolism, including the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). In inflammatory conditions, macrophages and neutrophils can produce great amounts of ROS and RNS to kill pathogens and remodel tissues. ROS and RNS can damage DNA in bystander cells, and macrophages themselves, and must be repaired. Ataxia telangiectasia mutated (ATM) is a kinase that responds to double-strand DNA breaks by phosphorylating key targets for repair, including KAP1 and H2A.X histone variant—common markers of DNA damage. ATM deficiency has recently been shown to trigger cGAS and STING innate immune pathways in macrophages through unrepaired DNA lesions, leading to inflammatory cytokine production and enhanced immunity to pathogens. Considering the growing associations between DNA damage and inflammatory pathways, we asked whether tissue resident macrophages respond differentially to DNA damage that would correspond to appropriate tissue specific outcomes. We find that alveolar, splenic, and peritoneal macrophages exhibit disparate levels of phosphorylated KAP1 after DNA damage, with only 40-50% of alveolar macrophages responding compared to 85-90% of peritoneal macrophages. This may be an outcome of differential ATM expression linked to tissue resident identity, as peritoneal macrophages have the highest levels of ATM mRNA. We propose that the control of ATM expression allows some tissue resident macrophages to respond differentially to DNA damage to achieve their outcomes.

P10. "Development and Application of an ***In Vitro*** Model of CD8+ T Cell Exhaustion"

Jennifer Wu, E. John Wherry

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

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

In our model, CD8 T cells are repeatedly stimulated ***in vitro*** with LCMV peptide (via presentation by dendritic cells) in the presence of low-dose IL-2. This method of stimulation is sufficient to induce three main modules of the

exhaustion phenotype: 1) high expression of inhibitory receptors, 2) certain aspects of the exhaustion-associated transcription factor signature, and 3) decreased effector cytokine production. Furthermore, these ***in vitro*** stimulation conditions are sufficient to commit these cells to an exhaustion lineage: when transferred into infected mice, they maintain high IR expression ***in vivo*** and preferentially persist and proliferate in chronic (as opposed to acute) infection, as has been previously demonstrated for bona fide exhausted CD8 T cells. Although further transcriptional and epigenetic validation of this ***in vitro*** model remains to be completed, we envision that this model will not only further our understanding of the biology of CD8 T cell exhaustion but can also be used to identify pathways and therapeutic targets that can potentially translate into novel and precise clinical treatments for cancers and viral infections.

P11. "IL-27-mediated regulation of inhibitory receptor expression"

Jonathan DeLong, Christopher Hunter

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” during chronic inflammation. TCR stimulation is known to drive expression of these molecules, but the role of cytokines in this process is only beginning to be understood. IL-27 is an IL-6/IL-12 family cytokine that is important in preventing immune hyperactivity during infection. Transcriptional profiling from our lab 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. I am using infection with the protozoan parasite Toxoplasma gondii to examine the in vivo relevance of these findings. Future studies will examine the role of these inhibitory receptors in suppressing immunopathology in this model.

P12. "IL-33 potentiates the inflammatory response to Toxoplasma gondii"

Joseph Clark, Jeongho Park, Christoph Konradt, Maxime Jacquet, Christopher Hunter

Mice deficient in the IL-33 receptor ST2 are susceptible to infection with the protozoan parasite Toxoplasma gondii, but how IL-33 promotes resistance to infection remains elusive. Previous reports have attributed this susceptibility to aberrant skewing of the immune response away from type 2 immunity, with which ST2 has been classically associated, toward an excessive type 1 response and consequent lethal immune pathology in the central nervous system (CNS). Although expression of ST2 by tissue- resident Tregs supports a possible deficiency in immune regulation, ST2-knockout mice were also found to have higher parasite burdens in the CNS, suggesting a potential defect in the inflammatory response. In recent years, a direct role for IL- 33/ST2 in enhancing type 1 responses has been appreciated, leading us to reconsider the function of IL-33 in the response to T. gondii. Using whole organ ***in vitro*** antigen recall assays of spleen, bone marrow, and brain tissues, we found that addition of exogenous IL-33 to Soluble Toxoplasma Antigen (STAg)-restimulated cultures enhanced interferon gamma production during both acute and chronic infection. Further, we observed that IL-33 enhanced the proliferation and differentiation of restimulated parasite-specific CD4+ and CD8+ effector T cells in vitro. To confirm the physiological relevance of our system, we used IL-33 reporter mice to confirm in vivo expression of IL-33 in intact tissues relevant to T. gondii infection, including the meninges and cervical lymph nodes. As IL-33 is expressed at high levels in many tissues, including the central nervous system, and is released during tissue damage caused by infections such as T. gondii, these results suggest that IL-33 enhances the inflammatory response to T. gondii and consequently control of this parasitic infection.

P13. "A systems biology approach to assess ubiquitin-mediated proteomic changes during T cell activation"

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

T cells participate in protection against pathogen invasion and cancer proliferation but their improper regulation can cause devastating autoimmune disease. When T cells encounter an antigen-presenting cell, T cell receptor (TCR) stimulation initiates a downstream signaling cascade that ultimately induces proliferation and differentiation into various effector cell populations. Ubiquitination acts within the TCR pathway to regulate T cell activation. However, the molecular targets and functional consequences of ubiquitination, within the context of TCR signaling, are not well understood.

We use a systems biology strategy to elucidate the role of ubiquitination in TCR signaling by quantifying changes in the 1) ubiquitin proteome 2) whole cell proteome and 3) transcriptome of mouse primary T cells during ***in vitro*** TCR stimulation. We identify ubiquitin substrates and measure the change in ubiquitination and the associated protein and transcript levels in response to TCR stimulation. Integration of the proteomics and transcriptomics data allows us to consider the effects of ubiquitination within the context of total protein abundance and transcript expression and thereby generate predictions of substrate-specific functional consequences of ubiquitination events in response to TCR stimulation. Interestingly, we find subsets of proteins for which an increase in ubiquitination does not coincide with a decrease in total protein abundance, which suggests that ubiquitination may be regulating fates beyond degradation.

Our data reveal many novel ubiquitin substrates and suggest that ubiquitination is acting within the TCR pathway to drive both degradative and non-degradative substrate fates and ultimately impact T cell activation. This study elucidates important aspects of T cell biology and provides insight into harnessing ubiquitination in T cells to treat autoimmune disease, promote defense against pathogens and advance cancer immunotherapies.

P14. "ECTV-encoded protein B22 restricts CD4+ T cell activation"

Katherine Forsythe, Laurence Eisenlohr

Orthopoxviruses encode many immunomodulatory proteins that profoundly interfere with various aspects of the immune system. Ectromelia (ECTV) is the orthopoxvirus that naturally infects mice, affording the opportunity to study viral-host interactions that have co-evolved. Importantly, disease progression closely mimics that of monkeypox and smallpox in humans. While inhibition of many immune cell types have been analyzed, to date ECTV interference with MHC-II mediated CD4+ T cell activation is not well defined. Here we show that ECTV infection of antigen presenting cells inhibits their ability to activate CD4+ T cells directly. However, the inhibitory mechanism is not straight forward as neither down-regulation of surface MHC-II nor soluble factors are responsible. A promising candidate protein for this inhibition is B22, which is located on the plasma membrane and has homologs in most virulent but not vaccine strains. Indeed, we have determined that B22 contributes substantially to ECTV virulence. Here we show that B22 is both necessary and sufficient to restrict CD4+ T cell activation, with experiments ongoing to elucidate the mechanism of inhibition.

P15. "Trib1 controls antiviral immunity by restraining T cell effector responses during chronic infection"

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

During an anti-viral immune response T cells exert effector functions to reduce viral burden. However, over the course of a persistent infection, T cells become exhausted and lose effector function leading to chronic viremia. While treatments targeting exhaustion have improved clinical outcome, the response is limited. Identifying how the effector response is regulated offers alternative strategies for bolstering the immune response during chronic disease. We identify Trib1 as a novel regulator of T cell effector responses during chronic infection. T cell specific deletion of Trib1 reduces viral burden and promotes T cell effector expansion and function. We also identify a new molecular mechanism regulating T cell activation whereby Trib1 restricts inflammatory signaling downstream of T cell receptor activation. Together, these results reveal a new mechanism regulating both CD4 and CD8 effector responses and highlight the potential for targeting regulators of these responses during chronic disease.

P16. "Trefoil factor 2 downmodulates Type I Inflammation in a LINGO3 dependent manner"

Kelly Zullo, Li-yin Hung, Yingbiao Ji, Karl Herbine, and De'Broski Herbert

Whether tissue reparative molecules regulate host protective immunity is poorly understood. Trefoil factors are epithelial-derived molecules that promote barrier integrity and regulate inflammation. TFF2 deficiency increases both epithelial cell permeability and interferon gamma (IFN-γ) secretion from CD4 and CD8 T cells. However, the mechanism(s) responsible for suppression of Type 1 inflammation via TFF2 remains unclear, due to the lack of a validated receptor. Our binding and co-localization studies show that TFF2 interacts with Leucine rich repeat Ig-like containing domain 3 (LINGO3). LINGO3$^{-/-}$ mice have increased IFN-γ secretion from CD4^{+} T cells at baseline and immunostaining studies demonstrate that LINGO3 expression is restricted to mucosal epithelia (CD45^{-}, EpCAM^{+}). Upon infection of Lingo3$^{-/-}$ mice with ***Citrobacter rodentium***, loss of LINGO3 enhances the control of bacterial replication burden more efficiently than WT counterparts. These data imply that a TFF2/LINGO3 axis limits Type I inflammation at the mucosa. Future studies will investigate whether LINGO3 shapes immunity through microbial-dependent or independent pathways.

P17. "Investigating CD4+ T cell development in the thymus"

Laura Chopp, Rémy Bosselut

Helper T lymphocytes (CD4+) are a critical component of adaptive immunity, yet the molecular mechanisms underpinning their development remain incompletely understood. CD4 T cells develop in the thymus from MHC Class II signaled CD4+CD8+ (double positive) precursor cells. In contrast, CD8+ cytotoxic T cells develop from MHC Class I signaled double positive precursor cells. While some transcription factors required for CD4 T cell development have been identified, how these factors function to establish the gene expression program characteristic of the CD4+ lineage is unclear. To identify differences between MHC Class I and MHC Class II signaled thymocytes, we conducted an unbiased, genome wide analysis of gene expression changes by RNAseq and of locus accessibility by ATACseq at multiple stages of intrathymic differentiation. Preliminary results from this unbiased search have defined candidate transcription factors, the function of which we are planning to analyze by gene knock-out and overexpression approaches.

P18. "Intratumoral Immune Activation Informs Rational CAR T Cell Design"

Lexus Johnson, Carl June, Andy Minn

Immune therapies have significantly improved outcomes for patients with poor prognosis in recent years, but are currently restricted to specific cancer types, and do not reach the majority of cancer patients. Thus, significant innovation is needed to extend the benefits of immune therapies to the majority of cancer patients. Our lab has recently identified the highly structured RNA 7SL as capable of stimulating immune response genes in tumor cells following secretion by neighboring fibroblasts. Thus, unshielded 7SL in the tumor microenvironment may represent a novel intratumoral DAMP that contributes to ICB responsiveness. We show that 7SL RNA is stimulatory to primary human DCs, and that increasing the amount of unshielded 7SL RNA present in the tumor microenvironment increases the frequency of intratumoral DCs and enhances T cell activation. Furthermore, this response is dependent on the signaling molecule MyD88, which is downstream of several TLRs, implicating innate immune recognition that leads to adaptive immune activation. In order to deliver this stimulatory RNA, we have designed a T cell-based system for delivering 7SL RNA directly to the tumor microenvironment. Using "synNotch" T cells we demonstrate that production of a model hairpin RNA activates primary human DCs and T cells ***in vitro***. In order test this approach ***in vivo*** we have developed a syngeneic system for CAR T cell administration in which B16 melanoma expresses human CD19 as a model neoantigen that can be targeted by murine CAR T cells. We have validated the efficacy of CAR T cells in this system, and will test the ability of synNotch CAR T cells to stimulate endogenous immune responses that control solid tumors more effectively. In total, this creates a framework for assaying the importance of a novel intratumoral DAMP while improving the immunogenicity of CAR T cells in solid tumors.

P19. "The Hedghehog pathway regulates the immunological properties of microglia"

Loic Dragin, Yohaniz Ortega-Burgos, Richa Kapoor, Miles M. Miller, Jorge I. Alvarez

Microglia are the resident myeloid cells of the Central Nervous System (CNS). They exert a patrolling role in the CNS to ensure homeostasis. During inflammation, the secretion of immune molecules such as cytokines and chemokines promotes in microglia a proinflammatory or anti-inflammatory program that ultimately leads to recruitment of immune cells and tissue repair. Thus, the immunological properties of microglia must be tightly controlled in order to orchestrate these processes without damaging the CNS. In Multiple Sclerosis (MS) and its animal model Experimental Autoimmune Encephalomyelitis (EAE), Blood-Brain-Barrier disruption leads to the extravasation of inflammatory mediators that stimulate the inflammatory phenotype of microglia and worsen disease severity. It has been shown that astrocyte-derived Sonic Hedgehog (Shh) promotes integrity and immunoquiescence of the BBB. Given the intricate interactions between microglia and other CNS cells, we hypothesized that Shh downregulates microglial activation. To explore this we assessed whether microglia i) expressed the components of the Hedgehog pathway; ii) responded to the presence of Shh iii) displayed a more activated phenotype when the Hh pathway was abrogated. We showed that human and murine microglia express key receptors (Patched-1 and smoothened (smo)) and transcription factor (Gli) of the Hh pathway. ***In situ*** imaging indicated that the Hh pathway is activated in microglia during EAE, and recombinant Shh decreases the secretion of proinflammatory molecules by microglia. Furthermore, pharmacological antagonism of the Hh pathway exacerbates EAE, which correlates with the formation of microglial clusters expressing proinflammatory markers. Finally, EAE induction in mice lacking the receptor Smo in Microglia (cx3cr1-cre $Smo^{c/c}$) leads to a chronic inflammatory process characterized by compromised production of IL-10 but increased GM-CSF expression. This phenotype correlated with exacerbated astrogliosis, demyelination and profound axonal pathology. Thus, our results provide evidence that Shh is a fundamental regulator of neuroinflammation.

P20. "Antigen-Specific Immunotherapeutic Vaccine for myasthenia gravis"

Jie Luo, Oliver Garden

Myasthenia gravis (MG) and its animal model, experimental autoimmune MG (EAMG), are caused by autoantibodies to the extracellular domain of muscle nicotinic acetylcholine receptors (AChRs). Autoantibodies to the cytoplasmic domain of AChRs do not cause muscle weakness because they cannot bind in vivo. The ideal MG therapy that would quickly and permanently suppress only the pathological autoimmune response to AChRs is currently unavailable. We have developed an antigen-specific immunotherapeutic vaccine for EAMG that involves immunizing rats with bacterially-expressed cytoplasmic domains of human muscle AChRs in adjuvants. Vaccine prevents onset of chronic EAMG, rapidly suppresses established EAMG, is potent, robust, long lasting, and safe because the therapeutic antigen cannot induce EAMG. The vaccine was initially developed using incomplete Freund's adjuvant, but works equally well with alum adjuvant routinely used for human vaccinations. Therapeutic mechanisms may involve a combination of antibody-mediated feedback suppression, Th2 polarization, and regulatory T and/or B lymphocytes-mediated active suppression.

P21. "Characterizing the role of long noncoding RNAs in innate antiviral immune responses"

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

Emerging viruses pose a substantial public health threat often causing severe symptomatic disease in endemic. Relevant examples within the last decade include Zika virus (ZIKV) and Chikunguyna virus (CHIKV), two phylogenetically disparate viruses which share tropism for epithelia, endothelia, and nervous tissue and have the potential to cause devastating clinical manifestations including neural abnormalities. Innate immunity is the first line of defense to endogenously control these pathogens. Although the proteins that comprise the innate response are well known, the nuanced regulatory mechanisms that are required to activate and/or repress the expression and function of these proteins are just beginning to be appreciated. Long noncoding RNAs (lncRNAs)

are a recently described regulatory species capable of altering transcription throughout cell biology. If and how lncRNAs contribute to innate antiviral immunity, a response characterized by the massive induction of inflammatory transcriptional programs, is not well understood. To address this, we performed an unbiased, high-throughput RNAi screen targeting 2500 lncRNAs in human brain microvascular endothelial cells (HBMEC) infected with CHIKV. Viral burden was measured using immunofluorescence probing for viral protein. An increase in viral load following depletion of a given lncRNA indicated that the target was potentially antiviral. In total, 12 lncRNAs were identified using these criteria without bias towards certain genomic regions or lncRNA biotypes. Initial analysis of these hits focused on defining 1.) baseline expression 2.) potential induction following infection and 3.) nuclear vs. cytoplasmic localization. In addition, we have performed a parallel screen using the same RNAi library in HBMEC infected with ZIKV. We identified 9 lncRNAs that impacted ZIKV infection. Interestingly, the ZIKV and CHIKV screens yielded distinct hits which may indicate differential antiviral responses or that the infectivity of these viruses is dependent on different host factors that are regulated by lncRNAs. Future studies will validate our preliminary findings from both the ZIKV and CHIKV screens, determine if these hits are active against other viruses in the same genera, and characterize the mechanism of action of these lncRNAs.

P22. "Effect of Natural Genetic Variation on the Chromatin Landscape During T Cell Development"

Naomi Goldman, Maria Fasolino, and Golnaz Vahedi

During T cell maturation in the thymus, progenitors commit to the T cell lineage by undergoing sequential cell fate decisions that are associated with specific changes in gene expression. These cell fate-specific gene expression programs are specified in part by alterations in chromatin accessibility via lineage-determining transcription factors (TFs). Genetic knockout studies have implicated a group of TFs as essential in the T cell differentiation program, but the chronological order of their interactions, as well as the extent and mechanism by which these factors collaborate, remains unknown. To address these questions, we utilized the natural genetic variation between two strains of mice (C57BL/6J (BL6) vs NOD/ShiLtJ (NOD), 5.8 million single nucleotide polymorphisms (SNPs)) as an ***in vivo*** mutagenesis screen to determine the effects of SNPs on chromatin accessibility, genome organization, and gene expression in double positive (DP) T cells. By examining the SNP-associated TF motifs that correlate with differentially accessible chromatin regions (via ATAC-seq), we can gain insight into potential T cell lineage-determining TFs. We have found a ~6 fold enrichment of SNPs in strain specific ATAC-peaks vs. strain similar ATAC-peaks, suggesting a role for these SNPs in perturbing chromatin accessibility. In addition, at SNPs in peaks in which BL6 DP T cells have more accessibility than in NOD cells, the motifs for Ets1, Runx1, and E2a are enriched. This implicates that the disruption of these motifs by SNPs in the NOD DP T cells may be contributing to the reduction in accessibility at these sites, suggesting that these factors play a role in establishing T cell-specific chromatin accessibility. To understand how these SNP-associated loci affect chromatin organization and gene expression, we have carried out H3K27ac-HiChIP experiments (an assay for H3K27ac-directed genomic architecture) and RNA-seq in both strains of mice. Through these studies, we aim to determine the identity and molecular consequences of lineage-determining TFs in T cell maturation.

P23. "CNS-intrinsic regulation of T cell inflammatory responses"

Richa Kapoor, Lara Cheslow, Miles Miller, Loic Dragin, Trini Ochoa, Jorge Ivan Alvarez

In MS and experimental autoimmune encephalomyelitis (EAE), astrocytes are the primary sensors of the cues leading to lesion formation. MS and EAE lesions are characterized by the perivascular accumulation of pathogenic T Helper-1 (Th1) and Th17 cells located in proximity to astrocytic processes expressing high levels of sonic hedgehog (Shh). Interestingly, blocking Shh signaling exacerbates the clinical outcome of EAE. Yet, how Shh regulates immune cell function under neuroinflammatory conditions remains poorly characterized. To explore this, we activated encephalitogenic CD4 T cells and found that Shh induces a significant decrease in their effector phenotype. To understand the effect of Shh on different Th subsets, we skewed T cells into the Th1, Th2 and Th17 phenotypes and found that Shh significantly increase the expression of IL-10 on Th2 and Th17 cells. As effector CD4+ T cells modulate neuroinflammation in EAE, we compromised Hh signaling in T cells using mice deficient on the Hh receptor smoothened (smo) (CD4-Cre; $smo^{c/c}$). EAE induction in these mice resulted in earlier onset of

disease and higher clinical scores. Histopathological analyses indicate that Hh-deficiency on the T cell compartment exacerbates immune cell infiltration, demyelination and axonal pathology as compared to controls. This phenotype was associated with increase expression of IFN-γ and GM-CSF as well as a profound reduction in IL-10 expression on Th cells. Interestingly, such effect was CNS centric as Hh-deficient CD4+ T cells in peripheral lymphoid organs expressed comparable levels of IFN-γ, GM-CSF, IL-17 and IL-10 than controls. Thus, Shh is a CNS-intrinsic signal antagonizing the inflammatory phenotype of encephalitogenic T cells. This study will provide us a better understanding of the mechanisms regulating T cell function within the CNS to improve the development of therapeutics antagonizing pathogenic responses and enhancing protective mechanisms.

P24. "Chronic infection skews B cell differentiation leading to impaired affinity maturation"

Ryan Staupe, E. John Wherry

Antibodies are important for control of serum viremia during viral infections. As such, many viruses capable of establishing persistence, HIV and HCV in humans and LCMV in mice, have evolved methods to subvert the humoral immune response and establish cronicity. While altered B cell compartments and ineffective antibody responses have been described the mechanism for dysfunction is not well understood. Using the LCMV mouse model of chronic infection, we have determined that chronic infection skews LCMV-specific B cell responses away from the germinal center fate and towards terminal plasma cell differentiation. This skewing results in impaired affinity maturation and may explain the delayed development of effective antibody responses seen during chronic infection. Additionally, we have found that high antigen loads characteristically seen during chronic infection drive the skewing towards plasma cell differentiation. Strategies to reverse or prevent skewing of B cell differention may help in the development of next-generation therapies or vaccines against high-impact human chronic viral infections.

P25. "Phenotypic and transcriptomic characterization of canine myeloid-derived suppressor cells"

Sabina Hlavaty, Michelle Goulart, Julia Wu, Eshita Sharma, Yu-Mei Chang, Dong Xia, John Gribben, Oliver A Garden

Myeloid-derived suppressor cells (MDSCs) are key players in immune evasion, facilitating tumor growth and metastasis. MDSCs accumulate under various pathological states, adopting one of two recognized phenotypes: polymorphonuclear (PMN)-MDSCs and monocytic (M)-MDSCs. Increased MDSC frequencies in the peripheral blood of cancer patients predict a negative prognosis. Studying the function of these cells in mice is not ideal, since murine models often do not reliably recapitulate human disease. Dogs develop spontaneous tumors that share many features of human cancers, making them an excellent model for cancer research. Our study aimed to characterize the phenotypic and transcriptomic signatures of MDSCs in the dog, defining for the first time polymorphonuclear and monocytic subsets in this species. MDSCs in the peripheral blood of tumor-bearing and healthy control dogs were defined as hypodense MHC class $II^-CD5^-CD21^-CD11b^+$ cells, with PMN-MDSCs defined as $CADO48A^+CD14^-$ cells, and M-MDSCs defined as $CADO48A^-CD14^+$ cells. In common with human studies, peripheral frequencies of PMN-MDSCs and M-MDSCs relative to total peripheral blood mononuclear cells were significantly different in dogs with cancer compared to healthy control dogs (PMN-MDSCs: $p < .001$; M-MDSCs: $p < .05$). Bioinformatic analyses further revealed that the transcriptomic signatures of PMN-MDSCs and M-MDSCs are distinct from each other and from those of polymorphonuclear and monocytic cells, respectively, but similar to the respective human populations. Our findings demonstrate for the first time that dogs have two distinct populations of MDSCs, characterized by specific phenotypic and transcriptomic signatures that share features of human MDSC subsets, validating the dog as a model for studying these cells in the context of cancer.

P26. "The steroid hormone 20-hydroxyecdysone (20E) transcriptionally regulates the midgut of Anopheles gambiae and Aedes aegypti to promote bacterial expansion"

Sarah Sneed, Michael Povelones

Anopheles gambiae and ***Aedes aeypgti*** mosquitoes are important vectors for human pathogens. The midgut is the first major tissue barrier bloodmeal-acquired pathogens encounter in the mosquito, therefore understanding the signals that regulate the midgut epithelium during a bloodmeal is critical. 20-hydroxyecdysone (20E) is a steroid hormone that is essential for reproduction and is produced after bloodfeeding. We found that the dramatic commensal expansion that normally occurs after a bloodmeal can be initiated with an injection of 20E in the absence of blood in both ***An. gambiae*** and ***Ae. aegypti***. We quantified and characterized the microbial communities expanding in the midgut after 20E injection and bloodfeeding using qPCR and metagenomic analyses, respectively. RNA-Seq was also performed on both of these mosquitoes to identify midgut-specific transcriptional targets of 20E. We hypothesize that a subset of targets found in both mosquitoes are directly responsible for a tolerating signal that allows the bacteria to expand post-bloodmeal and may simultaneously increase susceptibility of the midgut epithelium to pathogen infection.

P27. "Role of FBXW7 transcript variants in human B cells"

Scarlett Yang, Andrei Thomas-Tikhonenko

Traditionally, B cell development is thought to be orchestrated by expression of various transcription factors (TF). However, recent developments indicate that the underlying mechanisms are more complex and might include expression and function of alternative transcripts. For example, E2A, a TF with two isoforms regulates early B cell differentiation and class switching. However, the extent to which transcript variants contribute to human B cell development, humoral response, and neoplastic transformation is not clear. To examine this, I begun three lines of research: 1. To isolate various progenitor and B cell subsets (early, pro, pre, immature) from bone marrow (BM). 2. To isolate malignant B cells from primary B-Cell Acute Lymphoblastic Leukemia (B-ALL) patient samples. 3. To isolate mature B cell subsets from tonsils and activate them via BCR ligation. All three approaches were followed by deep RNA-Seq and analysis with MAJIQ algorithm to identify transcript variants. BM-derived B cell subsets from different developmental stages were compared to each other. B-ALL samples were compared to their normal counterparts. Results from activated B cells were compared to baseline. My results indicate that among hundreds of genes expressing alternative transcripts in all three experimental settings, one gene common for all three sets is the F-box and WD Repeat Domain Containing 7 (FBXW7). FBXW7 encodes an E3 ubiquitin ligase that targets substrates to proteasomal degradation. There are three coding isoforms of FBXW7, alpha, beta, and gamma. My findings suggest: 1. The transition from early progenitor to pro-B is accompanied by the alpha-to-beta isoform switch, which is then reversed at later stages. 2. B-ALL samples have differential FBXW7 alpha-to-beta ratios compared to normal counterparts. 3. Activation of naive B cells leads to alterations in the 5' non-coding exon usage. I hypothesize that various FBXW7 isoforms target distinct protein substrates for degradation and achieve temporal regulation of gene expression in B cell development in a physiological setting and can play a pathological role in neoplastic transformation. Future research efforts will be devoted to defining functions of various FBXW7 isoforms in normal B cell development and malignant transformation. Findings from this project could allow development of therapeutic approaches targeting B cell diseases.

P28. "ERM-deficient T cells exhibit defects in TCR signaling and trafficking to lymphoid organs."

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Ezrin, radixin, and moesin (ERM) proteins create specialized membrane subdomains by linking phosphatidyl inositol lipids and protein binding partners in the plasma membrane to the underlying actin cortex. In humans, mutations in ERM proteins cause severe immunodeficiency characterized by lymphopenia and poor T cell proliferation. To investigate the role of ERM proteins in T cell function, we generated mice with T cells deficient in ezrin and moesin, the two ERM family members expressed by T cells. While T cell development is normal, these mice exhibit elevated numbers of splenic T cells, reduction of T cells in peripheral lymph nodes and a near

absence of T cells in the blood. B cells (which lack moesin in our mice) show a similar tissue distribution. Analysis of T cell activation reveals that CD69 upregulation, IL-2 production and proliferation are impaired. Signaling studies reveal defects at the level of PLCg1 activation and calcium influx. Paradoxically, however, DAG-dependent MAP kinase signaling is elevated. In addition to defects in TCR signaling, ERM-deficient T cells exhibit enhanced integrin- dependent adhesion and clustering. In vivo, adoptive transfer experiments show that T cell trafficking is abnormal, and point to defects in entry into lymph nodes and egress from the spleen. Studies are underway to test the involvement of integrins and S1P receptors in the observed T cell trafficking defects. Taken together, these findings support a dual role for ERM proteins in T cell trafficking and TCR signaling.

P29. "***Leishmania***-specific skin resident CD4 T cells are formed from recently activated effector T cells"

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Tissue-resident memory T cells (Trm) are critical components of protective immunity against a variety of pathogens. The majority of studies have focused on Trm cells at the site of infection or immunization, where inflammation promotes T cell recruitment. In contrast, few studies have focused on how Trm cells gain access to non-inflamed sites, an important issue for designing vaccines to target these cells. In mice that have resolved a primary infection with ***Leishmania***, skin-resident memory CD4 T cells have recently been shown to provide protection against challenge at sites distant from the initial infection site. This provides a model to determine when and how CD4+ Trm cells enter non-inflamed skin. We found that while ***Leishmania***-specific CD4 T cells enter the site of infection within a few hours, T cells were not found in non-inflamed skin distant from the primary infection site until 2 weeks post infection, and continued to enter the non-inflamed skin for at least 5 weeks. However, using parabiosis of naïve mice and immune mice which have resolved infection, we found that ***Leishmania***-specific CD4 T cells present in the immune partner could not enter the non-inflamed skin of the naïve partner. In contrast, upon re-challenge of the parabionts, these CD4 T cells re-gained the ability to enter the non-inflamed skin of the naïve parabiont. To understand what allows entry of CD4 T cells into non-inflamed skin sites, we examined P- and E- selectin ligand (P&ESL) expression, and found that the CD4 T cells capable of entering non-inflamed skin sites expressed high levels of P&ESL. To further characterize these cells, we examined their proliferation, and found that the cells entering non-inflamed skin sites have recently proliferated, suggesting that only activated effector T cells gain entry to non-inflamed skin. Combined, these data demonstrate that recently activated effector CD4 T cells, but not memory CD4 T cells, are capable of entering non-inflamed skin sites, and suggest that P&ESL expression plays a role in this process. Future studies will examine if P&ESL expression is required for entry of CD4 T cells into non-inflamed skin sites, identify what other factors are involved, and determine what promotes the retention of Trm cells in the skin. This work will be a critical contribution to the development of vaccines targeting the generation, migration, and retention of pathogen-specific resident memory T cells to relevant tissue sites.

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