Friday, October 9

8:45 – 9:00AM

Welcome
https://primetime.bluejeans.com/a2m/live-event/dywrywue

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

9:00 – 10:00 AM

Session I
IMMUNOGENETICS
https://primetime.bluejeans.com/a2m/live-event/dywrywue

9:00 – 9:20am S1A. Davia Blake (Mentor: Kristen Lynch, PhD)
"CD28 regulation of alternative splicing events in primary human CD4 T cells"

9:20 – 9:40am S1B. Naomi Goldman (Mentor: Golnaz Vahedi, PhD)
"Exploring the mechanisms that establish the chromatin state of T cells"

9:40 – 10:00am S1C. Rebecca Glynn (Mentor: Craig Bassing, PhD)
"RAG DNA cleavage-induced repression of Rag1/2 transiently inhibits additional Igkappa rearrangements to enforce Igkappa allelic exclusion"

10:15 – 11:15 AM

Session II
CELL DEATH IN THE IMMUNE SYSTEM
https://primetime.bluejeans.com/a2m/live-event/dywrywue

10:15 – 10:35am S2A. Neha Nataraj (Mentors: Igor Brodsky, PhD and Sunny Shin, PhD)
"Defining Mechanisms of Extrinsic Apoptosis in Human Macrophages"

10:35 – 10:55am S2B. Trini Ochoa (Mentor: David Allman, PhD)
"Newly-formed plasma cells are more sensitive than long-lived plasma cells to bortezomib-induced mitochondrial-mediated apoptosis"

10:55 – 11:15am S2C. Rina Kim (Mentor: Robert Vonderheide, MD, DPhil)
"Role of Ferroptosis in Myeloid Derived Suppressor Cells in the Tumor Microenvironment"

11:30AM – 12:00 PM

Poster Pitch Blitz
https://primetime.bluejeans.com/a2m/live-event/dywrywue

12:00 – 2:00 PM

Poster Session & Lunch
*Note – Use the individual's BlueJeans link in order to visit their poster. Return to the main event link at 2PM.

12:00 – 1:00pm

- Daniel Aldridge - https://bluejeans.com/2107583090
- Brittney Allyn - https://bluejeans.com/2983702601
- Claudia Arevalo - https://bluejeans.com/8500377560
- Megha Basavappa - https://bluejeans.com/9186753918
- Gregory Chen - https://bluejeans.com/9854606560
- Michelle Cully - https://bluejeans.com/1174186422
- Bonnie Douglas - https://bluejeans.com/9465766508
- Marisa Egan - https://bluejeans.com/9371919108
- Megan Frederick - https://bluejeans.com/2857467499
- Brian Gaudette
- Jason Goldsmith - https://bluejeans.com/2352437002
- Jamal Green - https://bluejeans.com/5113265453/
- Andrew Hart - https://bluejeans.com/7783784644
- Samantha Kelly - https://bluejeans.com/8420918653
- Jennifer Londregan - https://bluejeans.com/5768007440

1:00 – 2:00PM

- Patrick Lundgren - https://bluejeans.com/3410387462
- Nawar Naseer - https://bluejeans.com/7098530622
- M.Betina Pampena - https://upenn.bluejeans.com/9137480163
- Joseph Perry - https://upenn.bluejeans.com/8854950097
- Indira Rao - https://bluejeans.com/7318711354
- Michael Scaglione - https://bluejeans.com/5525937164
- Lindsay Shallberg - https://bluejeans.com/7410496818
- Stacy Thomas - https://bluejeans.com/2726422857
- Luke Turner - https://bluejeans.com/3716569870
- Ashley Vanderbeck - https://bluejeans.com/6697250225
- Andrea Wong - https://bluejeans.com/3366595886
- Jennifer Wu- https://bluejeans.com/1458713519
- Scarlett Yang - https://bluejeans.com/8791567916
- Qin Zhu - https://bluejeans.com/2348518437

2:00 – 3:00 PM **Session III**
MICROBES AND PATHOGENS I
https://primetime.bluejeans.com/a2m/live-event/dywrywue

2:00 – 2:20pm S3A. Sophia Reeder (Mentor: David Weiner, PhD)
"Synthetic DNA Vaccines Adjuvanted with pIL-33 Drive Liver-Localized T Cells and Provide Protection from Plasmodium Challenge in a Mouse Model"

2:20 – 2:40pm S3B. Rina Matsuda (Mentor: Igor Brodsky, PhD)
"Inflammatory monocytes enhance control of Yersinia pseudotuberculosis by intestinal pyogranulomas"

2:40 – 3:00pm S3C. Joseph Clark (Mentor: Christopher Hunter, PhD)
"IL-33 promotes IL-12-dependent innate immunity to Toxoplasma gondii"

3:15 – 4:15 PM **Session IV**

MICROBES AND PATHOGENS II
https://primetime.bluejeans.com/a2m/live-event/dywrywue

3:15 – 3:35pm	S4A.	Eileen Goodwin (Mentors: Scott Hensley, PhD and Laurence Eisenlohr, VMD, PhD) “Effects of serum antibody on *de novo* B cell responses to influenza”
3:35 – 355pm	S4B.	Sarah Maddux (Mentor: Michael Silverman, MD, PhD) “The role of microbe-induced, early life Tregs in type I diabetes protection”
3:55 – 4:15pm	S4C.	Megan Clark (Mentor: Jorge Henao-Mejia, MD, PhD) “Exploring miR-Peptide-microRNA axes in innate immunity”

4:30 – PM

Keynote
Kate Fitzgerald, PhD

Professor of Medicine, Director of the Program in Innate Immunity and The Worcester Foundation Chair in Biomedical Sciences.
University of Massachusetts Medical School
Division of Infectious Disease & Immunology

"Mechanisms limiting Inflammatory Cell Death"

S1A. "CD28 regulation of alternative splicing events in primary human CD4 T cells"

Davia Blake, Caleb Radens, Kristen Lynch

Alternative splicing consists of exons that are selectively either included or excluded from the mature mRNA transcript. Previous studies have demonstrated that ~10-15% of alternatively spliced genes undergo signal-induced changes in isoform abundance in CD3/CD28 activated primary human CD4+ T cells. CD28 enhances various signaling events downstream of the TCR, however the contribution of CD28 signaling with splicing changes has not been investigated. Here we test the hypothesis that CD28 exerts some of its functional impact through the enhancement of splicing changes in T cells. We utilized poly(A) RNA-seq and the MAJIQ alternative splicing algorithm to analyze splicing events of human CD4+ T cells stimulated with CD3, a component of the TCR receptor, with or without CD28. Consistent with previous studies, we identified approximately 400 and 1,000 significant splicing events induced by CD3/CD28 stimulation at 8 and 48 hours of culture, respectively. Alternative splicing changes induced by CD3 and CD3/CD28 stimuli are highly correlative, however, approximately 23% of alternative splicing changes are further enhanced with additional CD28 costimulation at 8 hours of culture. This enhancement drops to 8% of alternative splicing events by 48 hours. In addition, a splicing event within the Casapse-9 transcript was validated to be regulated by CD28 costimulation. The skipping of exons 3-6 in Caspase-9 has been shown to enhance survival in cancer cells, and we hypothesize similar functions in T cells. We later plan to elucidate mechanisms of splicing changes in the context of CD28 costimulation and the functional consequences of such events, including Caspase-9.

S1B. "Exploring the mechanisms that establish the chromatin state of T cells"

Naomi Goldman, Aditi Chandra, Maria Fasolino, and Golnaz Vahedi

Cell fate-specific gene expression programs are established in part by alterations in chromatin accessibility via the action of lineage-determining transcription factors (TFs). Work in our lab has recently identified that the transcription factor TCF-1—integral for normal thymic development—targets and is essential for the opening of repressed chromatin in T cells1. However, the mechanism through which TCF-1 acts at enhancers in order to regulate genes during T cell development is unknown. To characterize the role of TCF-1 in the establishment of the T cell epigenome, I immunoprecipitated TCF-1 in thymocytes followed by a mass spectrometry (MS) analysis to identify interacting proteins. Previous work has shown that exogenous expression of TCF-1 in NIH3T3 fibroblasts leads to a gain in chromatin accessibility at T cell regulatory regions that are normally silent in fibroblasts. Given this observation, I have developed a system to validate candidate interacting proteins from my MS results. I have utilized CRISPR/cas9 to knock down candidates in fibroblasts prior to TCF-1 transduction followed by RNAseq and ATAC-seq to assess gene expression and chromatin state. Analysis of these results will allow me to determine if other factors are required to enable TCF-1 to modulate the chromatin state. These studies aim to provide mechanistic insight into how transcription factors work to establish cell identity in addition to adding to our understanding of how cell fate might be reprogramed at will for therapeutic purposes.

S1C. "RAG DNA cleavage-induced repression of Rag1/2 transiently inhibits additional Igkappa rearrangements to enforce Igkappa allelic exclusion"

Rebecca Glynn, Craig Bassing

V(D)J recombination is regulated such that lymphocytes assemble and express antigen receptor genes from one allele (allelic exclusion). One mechanism by which allelic exclusion is thought to be enforced is via DNA double strand break (DSB)-induced transient inhibition of recombination once one allele is cleaved by the RAG1/2 (RAG) recombinase. In pre-B cells, RAG-induced DSBs at Igkappa loci signal via ATM to repress Rag1/2 transcription, inhibit accessibility of Igkappa loci, and transiently suppress Vkappa recombination. We hypothesize that ATM-mediated inhibition of recombination is critical to ensure allelic exclusion and suppress oncogenic Ig translocations. Fittingly, Atm-/- mice have higher frequencies of immature B cells with RAG DSBs at both Ig alleles and of mature B cells with biallelic expression of these genes or a translocation of one allele. However, given that ATM also promotes efficient DSB repair and activates cell cycle checkpoints, these Atm-/- phenotypes cannot be attributed directly to ATM-mediated inhibition of recombination. To more directly test our hypothesis, we studied mouse models lacking downstream effectors of ATM: i) SpiC, through which ATM suppresses Igkappa accessibility and ii) NFkappaB essential modulator (NEMO), through which ATM activates broad transcriptional changes. We show that the frequency of surface bi-allelic Igkappa expression is normal on SpiC-/- B cells but increased on Nemo-/- B cells. We demonstrate that NEMO is required for RAG DSBs at Igkappa to repress Rag1/2 transcription and to limit RAG cleavage of Igkappa alleles. Additionally, we find that NEMO is required for SpiC expression, such that NEMO loss disrupts ATM-mediated suppression of both Rag1/2 expression and Igkappa accessibility. Overall, we conclude that RAG DSB-induced, ATM-signaled repression of Rag1/2 expression enforces Igkappa allelic exclusion independent of ATM-stimulated DSB repair. Furthermore, our data show that inactivation of ATM-mediated repression of Igkappa locus accessibility alone is not sufficient to increase biallelic Igkappa expression.

S2A. "Defining Mechanisms of Extrinsic Apoptosis in Human Macrophages"

Neha Nataraj, Igor Brodsky, Sunny Shin

Yersinia can cause illness ranging from food poisoning to plague. Infection can progress to septicemia, where treatment options are limited and serious complications can result. Yersinia causes disease by suppressing critical defense responses in immune cells, including NF-kB signaling. We and others have demonstrated that Yersinia infection in murine macrophages blocks NF-kB signaling and leads to apoptosis that requires activation of RIPK1 and caspase-8. In mice, inhibition of RIPK1 activity prevents apoptosis and bacterial clearance, resulting in host death. Thus, RIPK1-kinase-induced apoptosis provides protection from infection in mouse models. However, significant differences exist between mice and humans in expression of proteins that regulate apoptosis. Mice possess one cell extrinsic initiator, caspase-8; humans and all other vertebrates possess two, CASP8 and 10. Furthermore, murine deficiency in caspase-8 or RIPK1 results in embryonic lethality, whereas humans lacking these components are viable but develop inflammatory disease and exhibit increased susceptibility to infections. These observations indicate a key gap in our knowledge of the human apoptosis pathways. Intriguingly, my data indicate that apoptosis in human macrophages following NF-kB blockade is RIPK1-

independent, unlike mouse macrophages, suggesting a completely novel mechanism of cell death. I hypothesize that human macrophages induce apoptosis in response to bacterial blockade of NF-kB signaling via a unique mechanism that requires Casp8/10, but not RIPK1. To test this hypothesis, I will generate a comprehensive panel of human cell lines lacking RIPK1, its kinase activity or specific interacting domains, as well as those lacking Casp8 and/or 10. I will subject them to Yersinia infection and test their ability to undergo apoptosis using assays we have developed. These studies will provide new insight into the molecular basis of apoptosis in human cells, which will aid the development of improved treatment options for bacterial infection.

S2B. "Newly-formed plasma cells are more sensitive than long-lived plasma cells to bortezomib-induced mitochondrial-mediated apoptosis"

Trini Ochoa, Brian Gaudette, David Allman

Depletion strategies against malignant plasma cells or against plasma cells that produce donor-specific alloantibodies after organ transplantation have mainly utilized proteasomal inhibition (PI) to induce a terminal unfolded protein response (UPR) that results in apoptosis. However, no studies to date have evaluated the different survival capacities of newly-formed (NFPCs) and long-lived plasma cell (LLPCs) subsets to PI treatments. We have found that bortezomib primarily depletes NFPCs in the spleen and bone marrow during short-term treatments. Characterization of the treatment response revealed that LLPCs maintained significantly higher levels of the chaperone protein Bip, showcased decreased expression of the pro-apoptotic protein Bim, and reduced expression of the transcription factor XBP1s; potentially indicating a reduced ER-stress response in LLPCs to short-term BTZ treatment. In addition, we have utilized a minimalistic in vitro system where we identified the BCL-2 inhibitor ABT-199 as an accelerator of apoptosis in LLPCs that did not result in decreased expression of intracellular IgK light-chain in the surviving cells. Interestingly, in vitro treatment with BTZ did not result in apoptosis of LLPCs, but led to the reduced expression of intracellular IgK light-chain. Future studies will define whether CHOP, the transcription factor thought to mediate PERK-mediated apoptosis during terminal UPRs, is responsible for apoptosis of NFPCs; and, we will test ABT-199 in vivo.

S2C. "Role of Ferroptosis in Myeloid Derived Suppressor Cells in the Tumor Microenvironment"

Rina Kim, Ayumi Hashimoto, Mohit Seghal, Yulia Tyurina, Andrew Kossenkov, Valerian Kagan, Yulia Nefedova, Robert H. Vonderheide & Dmitry Gabrilovich

In the tumor microenvironment (TME), myeloid-derived suppressor cells (MDSC)s function as an immunosuppressive shield that protects the tumor from the host's immune system. To effectively and selectively block the immunosuppressive activities of MDSCs to improve the success of current cancer immunotherapies, it is necessary to have a better understanding of MDSC development and function. MDSCs accumulate in cancer and adopt one of two recognized phenotypes: polymorphonuclear (PMN)-MDSCs or monocytic (M)-MDSCs. PMN-MDSCs are the most abundant (>75%) of MDSCs. To identify mechanisms regulating their maintenance and turnover that may be intricately tied to their accumulation, contributions of different cell death pathways were assessed. Our preliminary findings indicate that PMN-MDSCs spontaneously die by an iron-dependent form of non-apoptotic cell death termed ferroptosis during tumor progression. Interestingly, only tumor-associated PMN-MDSCs, but not peripheral (lymphoid organ,

tissue, or blood associated) PMN-MDSCs, undergo ferroptosis. Further investigation of this phenomenon may help us better understand differences in biology of MDSCs in the periphery vs. the TME. Current studies are focused on assessing the biological effect of the secretome and releasate of these cells undergoing ferroptosis as different forms of cell death may affect neighboring immune cells and skew the immune response toward anti-tumor immunity or tolerance induction. While the mechanistic basis and immunological consequence of this tissue-specific cell death of PMN-MDSCs remain unclear, this study is expected to address the gaps in our understanding of immune regulatory mechanisms governing ferroptosis.

S3A. "Synthetic DNA Vaccines Adjuvanted with pIL-33 Drive Liver-Localized T Cells and Provide Protection from Plasmodium Challenge in a Mouse Model."

Sophia M. Reeder, Emma L. Reuschel, Mamadou A. Bah, Kun Yun, Nicholas J. Tursi, Kevin Y. Kim, Jacqueline Chu, Faraz I. Zaidi, Ilknur Yilmaz, Robert J. Hart, Benjamin Perrin, Ziyang Xu, Laurent Humeau, David B. Weiner, and Ahmed S. I. Aly

The need for a malaria vaccine is indisputable. A single vaccine for Plasmodium pre-erythrocytic stages targeting the major sporozoite antigen circumsporozoite protein (CSP) has had partial success. Additionally, CD8+ T cells targeting liver-stage (LS) antigens induced by live attenuated sporozoite vaccines were associated with protection in human challenge experiments. To further evaluate protection mediated by LS antigens, we focused on exported pre-erythrocytic proteins (exported protein 1 (EXP1), profilin (PFN), exported protein 2 (EXP2), inhibitor of cysteine proteases (ICP), transmembrane protein 21 (TMP21), and upregulated in infective sporozoites-3 (UIS3)) expressed in all Plasmodium species and designed optimized, synthetic DNA (synDNA) immunogens. SynDNA antigen cocktails were tested with and without the molecular adjuvant plasmid IL-33. Immunized animals developed robust T cell responses including induction of antigen-specific liver-localized CD8+ T cells, which were enhanced by the co-delivery of plasmid IL-33. In total, 100% of mice in adjuvanted groups and 71%–88% in non-adjuvanted groups were protected from blood-stage disease following Plasmodium yoelii sporozoite challenge. This study supports the potential of synDNA LS antigens as vaccine components for malaria parasite infection.

S3B. "Inflammatory monocytes enhance control of Yersinia pseudotuberculosis by intestinal pyogranulomas"

Rina Matsuda, Daniel Sorobetea, Igor Brodsky

In various infectious contexts, chronic immune stimulation induces the formation of granulomas: immune cell aggregates that are thought to sequester pathogens. Despite being a prominent feature of numerous infections, key gaps in our knowledge remain about the functional role of granulomas and their mechanisms of formation. Yersinia are bacterial pathogens that block immune cell function and induce granuloma formation in lymphoid tissues. Yersinia pseudotuberculosis (Yptb), closely related to plague-causing Yersinia pestis, causes self-limiting gastroenteritis and lymphadenitis. Following fecal-oral transmission, Yptb invades the intestinal mucosa and infects local lymphoid tissue including the Peyer's patches and mesenteric lymph nodes, then disseminates to distal organs. While granuloma formation is a hallmark of Yersinia infection of lymphatic organs, mechanisms of enteric control of Yersinia are relatively poorly understood. We observe, for the first time, granulomatous lesions throughout the small intestine during acute murine

Yptb infection. Live bacteria are abundant within granulomas but largely absent from non-granuloma intestinal tissue, suggesting that intestinal granulomas play a previously unappreciated role in mucosal control of Yptb. Paradoxically, bacterial virulence is necessary for granuloma formation, as avirulent Yptb lacking its type III secretion system and injected effector proteins does not induce intestinal granuloma formation. By histological and flow cytometric analyses, intestinal granulomas are highly enriched in neutrophils and inflammatory monocytes. Importantly, monocytes play a key role in bacterial restriction by intestinal granulomas, as monocyte-deficient Ccr2-/- mice exhibit a defect in granuloma-mediated control of Yptb, increased dissemination of bacteria to non-granuloma intestinal tissue and peripheral organs, and acute mortality following oral infection. Overall, this work will mechanistically define a previously unappreciated facet of the host immune response to Yersinia infection. Findings will provide new insight into poorly studied granulomatous disorders and potential therapeutic targets for the treatment of chronic disease.

S3C. "IL-33 promotes IL-12-dependent innate immunity to Toxoplasma gondii"

Joseph T. Clark, David A. Christian, Jodi A. Gullicksrud, Joseph A. Perry, Jeongho Park, Maxime Jacquet, Enrico Radaelli, Jonathan Silver, Christopher A. Hunter

Although mice that lack the IL-33R (ST2) are more susceptible to infection with the parasite Toxoplasma gondii, the role of IL-33 in innate resistance to this infection is unclear. In vivo, challenge with T. gondii is associated with increased numbers of stromal cells that express IL-33 while the degree of parasite replication correlates with levels of IL-33 release. The response to infection is accompanied by marked alterations in the Innate Lymphoid Cell (ILC) compartment characterized by the loss of ILC2 and the emergence of a subset of NK cells and ILC1s that were IL-33R+. The ability to compare the innate mechanisms of resistance in Rag-/- mice showed that loss of IL-33R resulted in reduced ILC responses and increased parasite replication. Furthermore, administration of IL-33 to Rag-/- mice resulted in a marked decrease in parasite burden associated with increased production of IFN-g and the recruitment and expansion of a population of Ly6chi CCR2+ inflammatory monocytes associated with parasite control. These protective effects of exogenous IL-33 were dependent on endogenous IL-12p40 and the ability of IL-33 to enhance ILC production of IFN-g. Together, these results highlight a protective role for IL-33 in innate resistance to T. gondii.

S4A. "Effects of serum antibody on de novo B cell responses to influenza"

Eileen Goodwin, Scott Hensley, Laurence Eisenlohr

In adults, immune responses to influenza are dominated by antibodies generated to prior exposures with a notable scarcity of new, strain-specific antibodies, a long-studied phenomenon termed "original antigenic sin". As most groups postulate that the lower activation threshold of memory B cells is responsible for their domination of secondary responses, the contributions of serum influenza antibody to this phenomenon have not been thoroughly examined. We investigated the effects of circulating influenza antibody on B cell responses in the absence of memory B cells. We found that mice with HA-specific antibody have fewer HA-specific B cells in germinal centers following vaccination, although germinal center formation is uninhibited. As B cell participation in germinal centers hinges on effective presentation of their cognate antigen to CD4 T cells, we assessed the impact of HA-specific antibody on antigen presentation by HA-specific B cells. Both antibody

against the same and against a non-competing epitope potently inhibit the presentation of MHC II-restricted epitopes by HA-specific B cells, suggesting antibodies can hinder B cell responses to novel epitopes of the same protein. This work begins to explore the underappreciated role of circulating antibody in suppressing de novo B cell responses.

S4B. "The role of microbe-induced, early life Tregs in type I diabetes protection"

Sarah Maddux, JB Lubin, Jamal Green, Tereza Duranova, Michael Silverman

Incidence of type I diabetes (T1D) has been rising rapidly in the last few decades. Although MHC-II haplotype is the most important risk factor for T1D, this increased rate of disease indicates that there are environmental factors that significantly impact pathogenesis. The microbiota has been identified as a key risk factor in both humans and animal models. Non-obese diabetic (NOD) mice that express an MHC-II E transgene (E□16/NOD mice) are completely protected from diabetes in a largely microbiota dependent manner. Early life immune-microbe interacts are key to this self-tolerance, as perturbations of the microbiota before or at weaning decrease protection but have no effect at later time points. We have identified the microbe Akkermansia muciniphila as a potent inducer of T-cell dependent antibody responses in E□16/NOD mice and found that it may also induce peripheral Tregs, which are key for T1D protection. Preliminary data suggests Tregs generated around weaning are be important for T1D protection, as depleting these Tregs increases insulitis in E□16/NOD mice and may hasten the onset of diabetes in NOD mice.

S4C. "Exploring miR-Peptide-microRNA axes in innate immunity"

Megan Clark, Walter Mowel, Jasmine Wright, Sam McCright, Jorge Henao-Mejia

MicroRNAs (miRNAs) are a class of small non-coding RNAs which begin as long primary miRNA transcripts (pri-miRNAs) that are processed in the nucleus, then exported to the cytoplasm for maturation. Recent work in plants has demonstrated that some pri-miRNA transcripts contain open reading frames upstream of the miRNA. Several of these transcripts can escape miRNA processing and produce highly conserved, small peptides known as miRNA-peptides (miPEPs). More importantly, these conserved miPEPs serve to reinforce the function of the miRNA encoded in same transcript, which demonstrates a novel, cooperative regulation of gene expression and cellular function. However, whether mammalian organisms produce miPEPs to reinforce microRNA function is unknown. Moreover, whether miPEPs contribute to the development of inflammatory disorders is unexplored. Since the innate immune pathways and pathogen defense mechanisms in plants are highly conserved across species, we hypothesized that miPEP-miRNA axes exist in mammals and are capable of regulating innate immune responses. To this end, we identified a single transcript encoding a highly conserved miPEP and miRNA, both of which are potently induced by TLR ligands in macrophages. Upon LPS stimulation, the miPEP is localized to the mitochondria, and the top target of the miRNA is a protein in the mitochondrial electron transport chain. Intriguingly, my preliminary data indicate that the miPEP and miRNA work in concert to remodel the subunit composition of the electron transport chain. Taken together, our data identify the first ever example of a miPEP-miRNA axis in mammals, which serves to alter mitochondrial function during inflammation.

P1. "The impact of IL-27 on monocyte responses to Toxoplasma gondii"

Daniel Aldridge, Jeongho Park, Anthony Phan, Christopher Hunter

https://bluejeans.com/2107583090

The cytokine IL-27 provides a critical mechanism to restrain immune hyperactivity during infection. During Toxoplasma gondii infection, the loss of IL-27 results in a lethal, CD4+ T cell mediated immune response as well as elevated inflammatory cytokine responses and systemic thrombosis. How these mechanisms mediate pathology, and possibly intersect with one another, is unclear. Recently, we observed that blockade of one of these elevated inflammatory cytokines, GM-CSF, leads to survival of infected IL-27 KO mice. GM-CSF can enhance monocyte and macrophage responses as well as contribute to immunothrombosis, suggesting that this may be a potential central mechanism by which IL-27 mediated protection is achieved. Additionally, we have observed that acutely the loss of IL-27 results in enhanced monocyte responses to infection. Monocytes do not express the IL-27 receptor, but long-term hematopoietic stem cells (LT-HSCs) do and can be skewed towards several differentiation pathways by IL-27. Therefore, we have begun to analyze if HSC development and monocyte phenotypes are impacted by IL-27 during the early stages of toxoplasmosis. This is being achieved through a combination of fluorescence imaging, high-dimensional flow cytometry, and scRNA-seq. We are complimenting this by determining the mechanisms by which GM-CSF mediates pathology in the absence of IL-27. This is done by blocking GM-CSF during infection of IL-27 KO mice and analyzing the immune parameters connected to pathology. Following this, the GM-CSF receptor will be selectively removed from potential cell types to determine if this rescues IL-27 deficient mice during infection. Together, these studies will enhance our understanding of cytokine driven pathologies and mechanisms of immune protection.

P2. "Elucidating Topological Regulation of Antigen Receptor Gene Assembly"

Allyn, B. M., Hayer, K., Nganga, V., Oltz, E., Bassing, C. H.

https://bluejeans.com/2983702601

The compaction of antigen receptor (AgR) loci is thought to be important for efficient assembly of broad AgR gene repertoires through recombination of V gene segments with distal D and/or J gene segments of the recombination center (RC). Compaction of mammalian genomes occurs through CTCF protein-mediated chromosome looping and compartmentalization of chromatin based on transcriptional state. AgR loci have many CTCF binding elements (CBEs) strewn amongst their V segments positioned in convergent orientation with a few CBEs flanking the RC. The field hypothesizes that V CBEs are vital to promote recombination of nearby V segments by bringing them into proximity with D/J segments through looping to the convergent RC CBEs. To test this hypothesis, we have mutated CBEs within the mouse TCRbeta locus. We find that deletion of the Trbv1 or Trbv14 CBE or inversion of the Trbv14 CBE lowers, but does not ablate, contact and rearrangement of flanking Vbeta segments with the RC. We also show that deletion of all RC CBEs alters Vbeta repertoire, skewing usage to Vbetas most proximal to the RC, but has no obvious effects on overall levels of Vbeta recombination or

alpha beta T cell development. Interestingly, we demonstrate that deletion of the Trbv1 promoter ablates transcription of Trbv1 as well as interactions and rearrangements between Trbv1 and the RC. Collectively, our data imply that compartmentalization might be the dominant mechanism for compaction and recombination of TCRbeta loci, with chromosome looping fine-tuningcompartmentalization-driven interactions to influence Vb repertoire.

P3. "Nucleoside-modified mRNA Vaccination generates Antibody Responses against 20 distinct Influenza Subtypes"

Claudia P. Arevalo, Marcus J. Bolton, Tyler Garretson, Kaela Parkhouse, Norbert Pardi, Scott E. Hensley

https://bluejeans.com/8500377560

There are 18 different subtypes of influenza A virus and 2 subtypes of influenza B virus that exist in animal reservoirs. Three of these 20 subtypes circulate seasonally and several others have pandemic potential. We tested a lipid nanoparticle-encapsulated, nucleoside-modified mRNA (mRNA-LNP) vaccine encoding 20 distinct Hemaglutinin (HA) proteins from each of the influenza subtypes. A single immunization with this vaccine generated antibody responses that recognize all antigens encoded by the vaccine. Furthermore, we found that the antibody responses also include those to broadly reactive and more conserved epitopes such as to the HA stalk domain. Importantly, mice survived an influenza challenge with a heterologous subtype not included in the vaccine. Our findings have implications for the development of a vaccine against more than just the four subtypes included in the seasonal influenza vaccines. Furthermore, they may provide a path to establishing immunity against a wider range of influenza subtypes.

P4. "The novel lncRNA ALPHA specifically targets chikungunya virus to control infection"

Megha G. Basavappa, Max Ferretti, Mark Dittmar, Megan Sullivan, Kanupriya Whig, Hui Shen, Julian Stoute, Fange Liu, David Schultz, Daniel Beiting, Kristen Lynch, Jorge Henao-Mejia, Sara Cherry.

https://bluejeans.com/9186753918

Chikungunya virus (CHIKV) is a recently emerged, mosquito-borne alphavirus which causes a severe, symptomatic disease characterized by chronic arthralgia. No vaccines or therapeutics currently exist to combat this virus. Canonical, type I interferon (IFN) signaling is an important line of defense against CHIKV. However, like many viruses, CHIKV has evolved mechanisms to effectively antagonize IFN responses. Despite this, acute infection is typically cleared; thus, alternative, complementary immune strategies must exist to compensate for virus-mediated immune abrogation. The nature of these non-canonical, anti-CHIKV pathways remains unclear. Long noncoding RNAs (lncRNAs) are a recently described class of regulatory RNAs capable of modulating many facets of nucleic acid biology including transcription and translation in a time- and context-specific manner. Recent work has implicated lncRNAs in the maintenance of immune cell homeostasis and the regulation of cytokine expression. However, the role of lncRNAs in antiviral immunity is only beginning to be appreciated. To directly identify antiviral lncRNAs, we performed an unbiased, high-throughput, RNAi screen targeting 2200 lncRNAs in human brain microvascular endothelial cells (HBMEC) infected with CHIKV.

Using an automated microscopy platform, we identified 9 lncRNAs that upon depletion, resulted in increased CHIKV infection in the absence of cytotoxicity. Further phenotypic characterization of these antiviral candidates revealed that the human-specific, long intergenic noncoding RNA, ALPHA, is localized in the cytoplasm, is transcriptionally induced upon infection, and displays high antiviral specificity for a subset of alphaviruses. Furthermore, ALPHA does not regulate IFN transcription suggesting a mode of action outside of classical innate immunity. Mechanistically, we found that ALPHA binds to viral genomic RNA and affects viral RNA replication supporting a direct mechanism of antiviral inhibition. Together, our findings reveal that lncRNAs can mediate directed, non-canonical antiviral immune responses against specific viral pathogens.

P5. "Characterizing the determinants of CAR T-cell persistence through high-dimensional molecular profiling of pre-manufacture T-cell populations"

Gregory M Chen*, Changya Chen*, Rajat K Das*, Chia-Hui Chen, Yang-Yang Ding, Yasin Uzun, Qin Zhu, Stephan A Grupp, David M Barrett#, Kai Tan#

https://bluejeans.com/9854606560

The adoptive transfer of Chimeric Antigen Receptor (CAR) T-cells has shown enormous promise in the treatment of B-cell Acute Lymphoblastic Leukemia (B-ALL), yet a significant proportion of patients fail to achieve long-term CAR T-cell persistence. Central to this challenge are the differences in the autologous T-cell starting material between patients. Here, we extensively characterize the pre-manufacture T-cells of 71 pediatric and young adult patients with B-cell malignancies enrolled to receive anti-CD19 CAR T-cell therapy. We characterize the composition and transcriptomic state of T-cell subpopulations through RNA-sequencing of sorted T-cells, revealing metabolic reprogramming and enrichment of proliferative and apoptotic pathways in differentiated T-cells. We design a mixed-effects interaction model to identify genes associated with clinical CAR T-cell persistence in a manner that controls for differences between T-cell subsets, and generate regulatory networks to capture the transcriptional underpinnings of T-cell states and clinical outcome. We validate our findings using internal and independent CAR T-cell datasets. These data may aid in improving patient prognosis and advancing the development of improved CAR T-cell therapies.

P6. "Lymphatic endothelial cell intrinsic IKKα in tertiary lymphoid organ formation and function"

Michelle D. Cully and Michael J. May.

https://bluejeans.com/1174186422

Tertiary lymphoid organs (TLOs) are ectopic accumulations of immune cells, specialized vasculature, and activated stroma that resemble a B cell follicle. They are induced by inflammation in a variety of diseases and can exacerbate disease or provide protection. TLOs in the lung for instance are protective in influenza infection. For this reason, driving TLO formation may be a valuable treatment opportunity, but the overall understanding of TLO formation and function remains incompletely understood. The non-canonical NF-κB pathway is required for secondary lymphoid organ development and recent evidence suggests that activation of this pathway in lymphatic endothelial cells (LECs) drives

lymphoid organogenesis. To study this in detail we generated mice lacking LEC-intrinsic IKKalpha, a central kinase in the non-canonical NF-κB pathway. These mice failed to development LNs and strikingly, contain aggregates of immune cells in the lungs that were otherwise healthy and undamaged. Further assessment of the immune cell accumulations revealed that they are lung-associated TLOs that we named spontaneous bronchus-associated lymphoid tissue (sBALT). Importantly, when these mice were infected with influenza virus they displayed remarkably improved morbidity and mortality compared with littermate controls. From these exciting preliminary findings, I hypothesize that targeting IKKalpha in LECs drives formation of sBALT that provides protection against respiratory infection.

P7. "Transgenic expression of a T cell epitope in Strongyloides ratti reveals that helminth-specific CD4+ T cells constitute both Th2 and Treg populations"

Bonnie Douglas, Yun Wei, Xinshe Li, Annabel Ferguson, Christopher Pastore, Li-Yin Hung, Jonathan R Kurtz, James B. McLachlan, Thomas J. Nolan, James Lok, and De'Broski R. Herbert

https://bluejeans.com/9465766508

Helminths are distinct from microbial pathogens in both size and complexity, and are the likely evolutionary driving force for type 2 immunity. CD4+ helper T cells can both coordinate worm clearance and prevent immunopathology, but issues of T cell antigen specificity in the context of helminth-induced Th2 and T regulatory cell (Treg) responses have not been addressed. Herein, we generated a novel transgenic line of the gastrointestinal nematode Strongyloides ratti expressing the immunodominant CD4+ T cell epitope 2W1S as a fusion protein with green fluorescent protein (GFP) and FLAG peptide in order to track and study helminth-specific CD4+ T cells. C57BL/6 mice infected with this stable transgenic line (termed Hulk) underwent a dose-dependent expansion of activated CD44hiCD11ahi 2W1S-specific CD4+ T cells, preferentially in the lung parenchyma. Transcriptional profiling of 2W1S-specific CD4+ T cells isolated from mice infected with either Hulk or the enteric bacterial pathogen Salmonella expressing 2W1S revealed that pathogen context exerted a dominant influence over CD4+ T cell phenotype. Interestingly, Hulk-elicited 2W1S-specific CD4+ T cells exhibited both Th2 and Treg phenotypes and expressed high levels of the EGFR ligand amphiregulin, which differed greatly from the phenotype of 2W1S-specific CD4+ T cells elicited by 2W1S-expressing Salmonella. Altogether, this new model system elucidates effector as well as immunosuppressive and wound reparative roles of helminth-specific CD4+ T cells. This report establishes a new resource for studying the nature and function of helminth-specific T cells.

P8. "Inflammasome Responses to Salmonella Typhimurium in Human Macrophages"

Marisa Egan, Nawar Naseer, Valeria Reyes Ruiz, Sunny Shin

https://bluejeans.com/9371919108

Salmonella enterica serovar Typhimurium is a Gram-negative enteric pathogen that relies on a type III secretion system (T3SS) to traffic virulence factors into the host. While these factors enable the intracellular survival of Salmonella within host cells, they also inadvertently facilitate host detection of Salmonella by cytosolic immune sensors. One such sensor is NAIP. Upon ligand recognition, NAIP interacts with NLRC4 to assemble the

NAIP/NLRC4 inflammasome. Inflammasomes are cytosolic, multiprotein complexes that mediate the activation of pro-inflammatory cytokines and pyroptosis. To investigate human inflammasome responses, we used macrophages derived from differentiating the human monocytic cell line, THP-1. Specifically, we generated NAIP and NLRC4 KO THP-1s using CRISPR/Cas9. Our data suggests that NAIP and NLRC4 are necessary for inflammasome responses to T3SS ligands. Moreover, NAIP and NLRC4 contribute to inflammasome responses during Salmonella infection of THP-1s. To interrogate other inflammasomes, we used a potent inhibitor of the NLRP3 inflammasome, MCC950. Only THP-1s deficient in NAIP or NLRC4 and treated with MCC950 displayed significantly diminished inflammasome responses upon Salmonella infection. Collectively, our data suggest that the NAIP/NLRC4 and NLRP3 inflammasomes synergistically respond to Salmonella in human macrophages. These findings inform our understanding of how Salmonella is detected by inflammasomes in human macrophages.

P9. "Different patterns of chromatin opening by hematopoietic pioneer factors"

Megan A Frederick, Meilin Fernandez Garcia, Gregory Donahue, Edgar Luzete-Montero, Naomi Takenaka, Kenneth S Zaret

https://bluejeans.com/2857467499

Pioneer factors can elicit local exposure of a nucleosome within chromatin and ultimately recruit co-regulators and remodelers to yield open chromatin sites seen in vivo. Yet how different pioneer transcription factors initially expose a targeted nucleosome in compacted chromatin structures is unclear. We used nucleosome arrays in vitro with a central nucleosome that can be targeted by the hematopoietic ETS factor PU.1 and the bZIP factors C/EBPα and C/EBPβ. Each class of factor can elicit targeted nucleosome exposure on linker histone-compacted arrays, but with different hypersensitivity patterns, as discerned from long-read nanopore sequencing. The DNA binding domains (DBDs) of PU.1 and C/EBPα are sufficient for mononucleosome binding but are much less efficient than the full-length proteins, which function cooperatively, in opening compacted chromatin. Thus, pioneer factors use their DBD to bind nucleosomes and other domains to elicit distinct patterns of target nucleosome exposure within compacted chromatin in vitro. Together our data give a mechanistic view of how transcription factors cooperate to disrupt chromatin structures to initiate DNA accessibility to additional regulatory factors.

P10. "The TIPE family of phospholipid transfer proteins regulate immune cell function by specifying the local excitation and global inhibition of signal transduction"

Jason R. Goldsmith*, Zienab Etwebi*, Ali Zamani, Mayassa Bou-Dargham, Ryan Hood, Chin Nien Lee, Mingyue Li, Ling Lu, Jiyeon Yu, Honghong Sun, Youhai H Chen

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https://bluejeans.com/2352437002

The TIPE (TNF-alpha-induced protein-eight-like) protein family is critical regulators of immune homeostasis, regulating basal and induced immune cell activation and differentiation, as well as cellular polarization and associated chemotaxis, with downstream effects impacting everything from infection and sepsis to colitis and tumor immunity. Until now, the mechanisms by which the TIPE family exert these profound

immunoregulatory effects have remained elusive. Here, we establish that the TIPE family of proteins regulate cellular local excitation and global inhibition (LEGI) of phospholipid-mediated signaling. TIPE proteins do this by changing their membrane vs cytoplasm-localization based on the amount of PIP2 in the membrane, in a family-member-specific manner. Furthermore, TIPEs use PIP3-dependent extraction of PIP2 as a sensor to detect regions of cellular activation, as PIP3 is only present at sites of local PI3K activation. At baseline, we found that TIPEs hold a portion of the cellular PIP2 in the cytosol, decreasing basal cellular activation by sequestering PIP2 away from PI3K. TIPEs can present this PIP2 to an activated PI3K, where it gets converted to PIP3 and re-inserted into the membrane, potentiating PI3K signaling at the site of activation. On the other hand, TIPEs without a PIP2 bound can interact with Akt and Rac and inhibit their function; yet at the active sites of signaling, TIPE-PIP2 complexes are present and thus this interaction would be lost , leading to local activation. TIPE2-Akt cytosolic interactions increase after immune cell activation, suggesting that TIPEs serve as global Akt inhibitors during active signaling. Together, this ongoing work provides for the first time a biochemical mechanism by which LEGI of phospholipid-signaling is enforced, with important implications for cellular homeostasis, particularly in relation to immune cell functions.

P11. "Major Histocompatibility Complex Shapes Early Life Microbial Events to Prevent Autoimmunity"

Jamal Green, Jean-Bernard Lubin, Sarah Maddux, Michael Silverman

https://bluejeans.com/5113265453/

Type 1 diabetes (T1D) is a debilitating autoimmune disease that affects millions. Unfortunately, the incidence of T1D is rising. The strongest genetic factor in T1D is the MHC class II locus. Some MHC haplotypes are associated with higher risk to T1D, while others provide dominant protection. Yet, the mechanism for protection remains unknown. Recent studies suggest that environmental factors, such as the microbiome, also contribute to the increasing incidence of T1D. While both genetic and environmental factors contribute to the risk of developing T1D, little is known of how MHC II genetic factors interact with microbial factors. The non-obese diabetic (NOD) murine model recapitulates many features of T1D in humans including the dominant protection associated with the MHC class II locus. NOD mice spontaneously develop T1D, but autoimmunity can be prevented by transgenic expression of the MHCII E allele (E-alpha-16/NOD mice). Recent studies suggest that the early-life microbiota is critical for this protection. To rigorously study the early-life microbiota, we developed a novel consortium of 9 culturable bacteria (PedsCom) that represent over 90% of the bacteria in pre-weaning diabetes-protected E-alpha-16/NOD mice. We are applying gnotobiotic techniques using the PedsCom consortium and genetic models of disease utilizing E-alpha-16/NOD mice to investigate the extent to which MHC II E expression impacts early-life events to shape microbial colonization by comparing colonization dynamics and humoral responses to commensal bacteria in the NOD and E-alpha-16/NOD mice colonized by the PedsCom consortia. In complementary experiments, we will determine if the PedsCom consortia of early-life microbes are sufficient to prevent T1D and whether peripheral regulatory T cells prevent T1D in E-alpha-16/NOD and NOD mice. We aim to identify immunomodulatory commensal bacteria and immune pathways that will be the basis for develop effective preventative therapies for T1D.

P12. “CD4 T cell dysregulation in systemic lupus erythematosus”

Andrew Hart, Andrew Wells, Terri Laufer

https://bluejeans.com/7783784644

In patients with systemic lupus erythematosus (lupus), CD4 T cell populations are aberrantly activated and differentiate into functional effector cells capable of supporting autoantibody formation and contributing to tissue pathology. TCR signaling, cytokine production, and homeostatic maintenance of CD4 T cell subsets are demonstrably altered in lupus, but the full extent and origins of T cell dysfunction are unclear. As CD4 T cells play an active role in disease pathogenesis and are targets of immune suppressants used in treatment, a better understanding of disease-driven T cell states is needed. We have identified a lupus-associated epigenetic and transcriptional phenotype among multiple PBMC-derived CD4 T cell subsets characterized by generalized hyper-accessibility of chromatin relative to healthy controls. ATACseq and RNAseq datasets indicate TNF and NFkB family signatures among altered epigenetic and transcription profiles. These data suggest the possibility that distinct underlying mechanisms of lupus etiology might converge on a shared CD4 T cell phenotype.

P13. “Notch2 signaling controls the expression of chemotactic receptors and integrins important for MZB cell positioning and migration”

Samantha Kelly[1,2,] Daniela Gómez Atria[2], Brian Gaudette[3], Eric Perkey[2], Léolène Carrington[2], Tanner Robertson[5], Anneka Allman[2], Chris Siebel[4], Janis Burkhardt[5], David Allman[3], Ivan Maillard[2].

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https://bluejeans.com/8420918653

The positioning of marginal zone B cells (MZB) within the marginal zone of the spleen is important for their function as a first line of defense against blood-borne pathogens. Previous studies supported the role of Delta-like1 (DLL1) Notch ligands and Notch2 receptors in cell fate decisions when MZB cells first develop from transitional B cells. We recently observed that continuous DLL1/Notch2 signaling is also necessary for MZB cells to remain positioned in the marginal zone. When Notch2 signals are blocked with anti-Notch2 antibodies, MZB cells leave the marginal zone and are seen in the red pulp by confocal imaging and in the blood after just 24 hours. RNA-seq of MZB cells after Notch2 blockade revealed profound changes in the expression of several chemotactic receptors and integrins relevant to the positioning of MZBs in the marginal zone. In addition, Notch2 blockade decreased expression of Myc and pathways associated with plasma cell differentiation. To further explore the role of Notch2 in MZB cell homeostasis, we examined the surface expression of chemotactic receptors and integrins by flow cytometry and the migration ability of MZB cells using transwell chemotaxis assays after Notch2 blockade. Notch-deprived MZB cells downregulate key receptors and integrins previously

shown to play a role in their positioning, such as CXCR5 and αLβ2 (LFA-1), while upregulating CXCR4 (whose ligand CXCL12 is abundant in the blood and red pulp). We speculate that decreased LFA-1 and/or increased CXCR4 signaling disrupt the anchoring of MZB cells in the blood-rich marginal zone. Altogether, our data indicate that Notch2 not only controls the development, but also the strategic positioning of MZBs through regulated expression of chemotactic receptors and integrins.

P14. "Stromal Notch ligands drive Notch2-dependent transdifferentiation of follicular B cells into marginal zone-like B cells in lymphopenic environments"

Daniela Gómez Atria, Brian Gaudette, Jennifer Londregan, Eric Perkey, Samantha Kelly, Burkhard Ludewig, Chris Siebel, Russell Ryan, Warren S. Pear, Bhaskar Srivastava, David Allman, Ivan Maillard

https://bluejeans.com/5768007440

In lymphopenic environments, B cells undergo homeostatic proliferation to maintain the size of the mature B lymphocyte pool. In the spleen, this pool is composed mainly of follicular B cells (FoB) which exist in organized follicles and participate in germinal center reactions; and marginal zone B (MZB) cells, that localize at the blood-red pulp interface to survey for blood-borne bacteria with the capacity to rapidly differentiate into plasma cells. To examine the factors that underlie the size and maintenance of the mature B cell compartment, we employed an adoptive transfer model of highly purified CTV-labeled B6-CD45.1 FoB cells into Rag2-/- mice. In a lymphopenic Rag2-/- environment, transferred FoB cells rapidly altered their phenotype to resemble MZB cells. As early as day 2 post-transfer, increased expression of IgM, CD21, CD1d and downregulation of IgD and CD23 was observed among transferred cells. These phenotypic changes were followed by a proliferative burst, as indicated by CTV dilution. Immunofluorescence microscopy of Rag2-/- recipients reveals that transferred CD45.1+ cells localized around CD169+ metallophilic macrophages that line the marginal sinus. In contrast, CD45.1+ cells recovered from lymphoid-replete B6 recipients maintained a FoB phenotype and remain CTV+, consistent with competition for an already full niche. Reminiscent of normal MZB development, FoB transdifferentiation into MZB-like cells was dependent on Dll1/Notch2 signaling, as systemic Notch2 blockade or genetic ablation of Delta-like1 (Dll1) Notch ligands on fibroblastic stromal cells in Rag2-/-Ccl19-Cre+Dll1f/f recipient mice blocked MZB transdifferentiation, as well as MZ localization and proliferation of transferred FoB cells. We are now investigating if transferred cells function as bona fide MZB cells in vivo and in vitro. Together, these findings highlight a previously unrecognized plasticity among mature FoB cells that challenges our understanding of B cell differentiation, and highlights the relevance of Notch signaling for the homeostatic maintenance of B cell populations.

ABSTRACTS | POSTERS 1:00 – 2:00 PM

P15. "Identifying regulators of Trem2: with a perspective on cardiovascular disease"

Patrick Lundgren, Hélène Descamps, Kirti Nath, Sam McCright, Leonel Joannas, Jorge Henao-Mejia, Christoph Thaiss

https://bluejeans.com/3410387462

Tissue resident macrophages are important regulators of diverse tissue functions in homeostasis and disease. Recent discoveries implicate the Trem2 receptor as a major immune signaling hub that senses tissue damage and promotes immune remodeling. Here, we have explored the dynamics of Trem2-expressing macrophages in atherosclerotic cardiovascular disease and performed preliminary experiments to identify inducers of Trem2 expression using a novel transgenic mouse. The identification of regulators of Trem2 expression may pioneer actionable targets whereby tissue physiology can be modulated by immunotherapy in disease.

P16. "Differential role of the NAIP/NLRC4 inflammasome in sensing Salmonella infection of human macrophages and intestinal epithelial cells"

Nawar Naseer, Marisa Egan, Valeria Reyes Ruiz, Sunny Shin

https://bluejeans.com/7098530622

Salmonella is a leading cause of diarrheal disease. Salmonella uses type III secretion systems (T3SS) to inject effectors into the host cell cytosol. These T3SSs enable Salmonella to invade and replicate within host cells such as intestinal epithelial cells (IECs) and macrophages. It is therefore critical to understand how IECs and macrophages, which serve both as targets of infection by Salmonella, as well as the first line of defense against invading enteric pathogens, are sensing and responding to infection. Host cells can recognize T3SS components via cytosolic receptors termed inflammasomes. One such inflammasome, termed the NAIP/NLRC4 inflammasome, recognizes bacterial T3SS components and flagellin. Inflammasome activation leads to proinflammatory cytokine release and cell death, alerting nearby cells of the infection and eliminating the pathogen's replicative niche. In mice, Salmonella infection triggers activation of the NAIP/NLRC4 inflammasome and this response is critical for protecting mice from Salmonella. In humans, both macrophages and IECs undergo inflammasome activation in response to Salmonella infection. Using CRISPR/Cas9 technology, we have found that human macrophages require NAIP/NLRC4 for maximal inflammasome responses, whereas human IECs undergo NAIP/NLRC4-independent inflammasome activation during Salmonella infection. Our findings highlight the differential role of the NAIP/NLRC4 inflammasome in different cell types.

P17. "Enforced lymphoid tissue retention of CD8+ T cells in SIV-infected rhesus macaques does not induce control of SIV plasma viremia"

M. Betina Pampena, Leticia Kuri-Cervantes, Sadia Samer, Meagan Watkins, Ronald S. Veazey, Katharine J. Bar, Brandon F. Keele, Miles P. Davenport, Mirko Paiardini, Michael R. Betts

HIV/SIV infected CD4+ T cells localize primarily to mucosal and lymphoid tissues (LT). Furthermore, LT are a major site for maintenance and subsequent recrudescence of the long-term HIV-reservoir. The role of cytotoxic CD8+ T cells is critical for control of HIV/SIV viremia. However, the most potent peripheral blood cytotoxic CD8+ T cells are rarely found in LT, indicating that they seldom have the opportunity to interact with infected CD4+ T cells. Here, we assessed the impact of LT CD8+ T cells on SIV immunopathogenesis by inhibiting cell egress from LT in viremic rhesus macaques (RM) using the lymphocyte migration inhibitor FTY720. We hypothesized that the retention of recirculating CD8+ T cells in LT may enable local differentiation into cytotoxic effector cells

with a subsequent impact on plasma viral load. Four RM were infected intravenously with SIVmac239, and treated with FTY720 daily from day 7 or 28 until day 90 post-infection. Separately, fourteen acutely infected RM were treated with antiretrovirals (since day 14 post-infection) for 6 months followed by treatment interruption while seven of them were receiving FTY720. We observed near complete redistribution of circulating CD8+ and CD4+ T cells into tissues within 28 days of FTY720 treatment. However, no beneficial effect was observed on peak viremia or set point during acute infection, or time and degree of viral rebound after therapy interruption. FTY720-enforced tissue retention promoted an increase in the frequency of SIV-specific CD8+ T cells in LN of FTY720-treated RM after antiretroviral treatment interruption. Nevertheless, the frequency of perforin+ granzyme B+ within LN SIV-specific CD8+ T cells remained low. These data indicate that simply increasing the number of CD8+ T cells in LT is insufficient to enable viral control in the SIV model. Moreover, enforced retention of CD8+ T cells in viremic tissues does not enable the acquisition of cytotoxic properties, suggesting that secondary signals not present in LT may be necessary to promote or maintain cytotoxic CD8+ T cell differentiation.

P18. "PD-L1 – PD-1 interactions limit effector Treg cell populations at homeostasis and during infection"

J.A. Perry, J.T. Clark, J. Gullicksrud, J. DeLong, L. Shallberg, B. Douglas, A. Hart, C. Konradt, J. Park, A. Glatman-Zaretzky, R. de Waal Malefyt, D.A. Christian, A. H. Sharpe, C.A. Hunter.

https://bluejeans.com/8854950097

While much is known about the factors that promote the development of diverse Treg cell responses, less is known about the pathways that constrain Treg cell activities in order to allow the development of effector T cell responses required for resistance to infection. The studies presented here reveal that at homeostasis there is a population of effector Treg cells that express PD-1 and that blockade of PD-L1, or loss of PD-1 results in increased Treg cell activity. In response to infection with the parasite T. gondii, the early production of IFN-γ results in widespread upregulation of PD-L1. Moreover, blockade of PD-L1, whole body deletion of PD-1, or lineage-specific deletion of PD-1 in Foxp3+ cells prevented the loss of the effector Treg cells but resulted in reduced pathogen specific CD4+ T cell responses during infection. Thus, at homeostasis basal PD-L1 expression constrains the pool of Treg cells, but during infection the upregulation of PD-L1 provides a mechanism to contract the Treg cell population required to maximize the development of pathogen specific CD4+ T cell responses.

P19. "The effect of altered lipid sensing on skin immunity to asymptomatic S. aureus colonization"

Indira N Rao, Neris Michel Enamorado Escalona, Djalma Lima Junior, Yasmine Belkaid

https://bluejeans.com/7318711354

The cellular constituents which make up our skin, the largest and outermost organ, are uniquely adapted to shield the host from numerous external challenges. While the surface of the skin fosters a diverse microbial metacommunity, the underlying tissue boasts a complex immunological arsenal. Epidermal keratinocytes and dermal lymphocytes

collectively orchestrate surveillance of skin commensals, resulting in non-inflammatory antigen-specific responses that promote host fitness while tolerating commensal persistence. Staphylococcus aureus is a predominant member of the skin microbiome as well as a leading opportunistic pathogen causing the majority of endogenous staphylococcal infections. Despite this pathogenic potential, cutaneous surveillance of and response to asymptomatic S. aureus colonization prior to infection remains largely unknown. A key factor contributing both to microbial colonization well as immune homeostasis is the metabolic landscape of skin. However, how host metabolic status affects immunity to asymptomatic staphylococcal colonization is unknown. We postulate that specific dietary lipids regulate the pathways underlying cutaneous immunosurveillance and host-microbe interactions. My preliminary data show that primary keratinocytes from C57Bl/6 mice treated in vitro with fatty acids upregulate production of key chemokines involved in recruitment of T cells to the skin. In the mouse model, I observe that even a short regimen of high-fat diet and concomitant S. aureus colonization enhance accumulation of type 17 innate and adaptive T cells in the skin, without causing inflammation. Surprisingly, the high fat diet also led to significantly enhanced S. aureus skin colonization, indicating that the upregulation of type 17 response does not inhibit colonization. Cutaneous lipids likely mediate skin immunity to asymptomatic S. aureus colonization. Delineating the role of lipid sensing in skin immunity to staphylococcal colonization is vital for developing targeted immunotherapies to moderate the evolutionary arms race between this furtive microbe and its sapient host.

P20. "The MLX family of metabolic transcription factors in immune cell activation"

Michael Scaglione, Will Bailis

https://bluejeans.com/5525937164

Immune cells have recently gained attention for their incredible dynamism in cellular metabolism. Upon activation, these cells radically rewire their metabolic networks to divert biochemical molecules needed for proliferation, differentiation, and cytokine secretion. Immune cells, especially tissue-resident cells, must also constantly sense and respond to changes in their metabolic environment that often accompany infection or changes in homeostasis. However, work is only starting to reveal the mechanisms by which immune cells translate information about their metabolic environment into their lineage- and tissue-specific functional programs.

My current work focuses on the integration of nutrient sensing by the MLX family of transcription factors into programs of immune cell activation and function. MLX family members directly sense glycolytic intermediates and divert these products into anabolic processes including de novo lipogenesis and nucleotide biosynthesis. These processes are highly leveraged by proliferative immune cells such as lymphocytes. Thus, I hypothesize that MLX supports lymphocyte activation by coordinating the engagement of anabolic metabolic networks necessary for proliferation. Preliminary data I have collected suggests that MLX activity may be required for the proliferation and function of CD4+ T cells.

Immune cells often function in unique tissue niches that vary widely in their metabolic content. Additionally, altered diet or metabolic disease can disrupt global organismal metabolism, shifting the immunologic response to infection or altered homeostasis. Recent

work suggests that MLX activity is dynamically modulated by a cell's internal metabolic state and external environment. I hypothesize that the MLX family is a key sensor of the metabolic microenvironment in immune cells, integrating information about sugar, lipid, and ketone body availability into changes in functional programming. I aim to test the role of MLX family members in linking changes in metabolic state to alterations in immune cell function using in vitro culture systems as well as in vivo dietary interventions.

P21. "Contribution of antigen stimulation to the heterogeneity of the CD8+ T cell response to infection"

Lindsey A Shallberg, Anthony T Phan, David A Christian, Joseph Perry, Daniel P Beiting, Christopher A Hunter

https://bluejeans.com/7410496818

In response to infection, initial TCR engagement of CD8+ T cells with cognate antigen results in the activation and expansion of pathogen specific T cells that give rise to various effector and memory phenotypes. Yet, during a protective response it has been difficult to assess the proportion of CD8+ T cells re-stimulated by cognate antigen at sites of infection and if re-encounter influences CD8+ T cell phenotype, function, or fate. Therefore, Nur77-GFP reporter OT-I T cells, which transiently express GFP when stimulated with SIINFEKL peptide, were paired with transgenic Toxoplasma gondii that express OVA to estimate how frequently pathogen specific CD8+ T cells re-encounter cognate antigen and the consequence of re-stimulation during acute and chronic toxoplasmosis. In vivo, the kinetics and levels of TCR stimulation in infected tissues correlated with parasite burden. In the brain, Nur77-GFP+ OT-I were transcriptionally distinct from Nur77-GFP- OT-I, with multiple transcription factors and gene sets being differentially expressed between the two populations. Adoptive transfer of Nur77-GFP-/+ OT-I from acutely infected mice into naive recipients resulted in formation of similar memory populations. These findings not only characterize the evolution of peripheral antigen stimulation during a chronic infection, but also underscore the importance of TCR signaling in generating a heterogenous CD8+ T cell response to infection while maintaining cell plasticity to generate memory T cell pools.

P22. "Liver macrophages play an anti-metastatic role in early pancreatic cancer"

Stacy K. Thomas, Jae W. Lee, Meredith L. Stone, Gregory L. Beatty

https://bluejeans.com/2726422857

Pancreatic ductal adenocarcinoma (PDAC) is the 3rd leading cause of cancer deaths in the United States. For most patients, metastatic disease is the main cause of morbidity and mortality. The liver is the most common site of PDAC metastasis. Our previous work demonstrated that a primary pancreatic tumor can induce the production of soluble factors, which in turn promote formation of a pro-metastatic niche in the liver. This pro-metastatic niche supports tumor cell seeding. Macrophages are a predominant component of this niche, yet their role in regulating metastatic seeding remains ill-defined. My preliminary data suggest that, in the absence of a niche, liver macrophages restrict tumor cell seeding in the liver. Meanwhile, published data shows that liver macrophages can promote tumor outgrowth after seeding has already occurred. Within the liver in the absence of a niche, Kupffer cells (KCs) are the dominant resident macrophage population. In contrast, bone marrow-derived macrophages (BMDMs) are recruited to the liver in the setting of

inflammation and accumulate in the liver during the formation of a pro-metastatic niche. It is currently unknown how each macrophage subset uniquely contributes to the metastatic process. However, my preliminary data suggest KC and BMDMs exist in distinct niches within liver metastasis, suggesting each cell type has a unique role in metastasis. Together these findings suggest that KCs have an inherent anti-metastatic capacity however, during cancer development, this biology is undermined by an influx of BMDMs into the liver that promote metastatic seeding and outgrowth. Future work aims to elucidate the roles of KCs and BMDMs in the liver in the presence and absence of a pro-metastatic niche.

P23. "NAD Salvage is a Novel Mediator of T Cell Activation"

Luke Turner*, Clémence Quériault*, James Davis, Alisa Sukina, Michael Scaglione, Kelly Rome, Janet Nguyen, Joseph Baur, Will Bailis

https://bluejeans.com/3716569870

An activated T cell's exit from quiescence is initiated by a series of signaling cascades that result in rapid cell proliferation and epigenetic remodeling. It is now appreciated that T cell activation also requires concurrent metabolic rewiring to support the biochemical demands of these processes. We have found that a central component of these early metabolic alterations is a marked expansion of cellular NAD+ levels. These changes are not only rapid, but occur at fold increases not observed in other tissues or cell types. In contrast to naïve T cells, activated T cells uniquely require a high rate of NAD+ synthesis. Strikingly this dependency is confined to the first two days following activation, despite continuous NAD consumption being observed after. We have thus sought to understand both the cellular requirements NAD+ synthesis as well as the molecular mechanism explaining this phenomenon. We have shown that NAD+ synthesis through the NAD salvage pathway is required for cell cycle entry, cytokine production, and glycolysis, but not for the induction of early markers of activation, such as CD69. We have further shown that the strength of T cell receptor (TCR) stimulation as well as co-stimulation directly determines the cellular NAD+ content in a dose dependent manner, as well as transcript levels of the rate limiting enzyme for NAD biosynthesis, Nampt. Interestingly, TCR and co-stimulation seem to regulate NAD+ synthesis through separate mechanisms, suggesting a novel metabolic mechanism for the regulation of T cell activation.

P24. "Fibroblastic stromal cells expressing Delta-like Notch ligands control extrathymic T cell development in mesenteric lymph nodes"

Ashley Vanderbeck, Samantha Kelly, Leolene Carrington, Frederick Allen, Gloria Jih, Anneka Allman, and Ivan Maillard

https://bluejeans.com/6697250225

Early T cell development is supported by signals mediated by the Notch1 receptor in T lineage progenitors and Delta-like4 (Dll4) ligand in thymic epithelial cells. Although T cell development is normally restricted to the thymus, extrathymic T cell development has been reported in athymic Foxn1nu/nu nude mice as well as during times of thymopoietic stress such as post-bone marrow transplantation (BMT). A population of CD4+CD8□ double positive T cell progenitors can be found in the mesenteric lymph nodes (MLN) in both athymic mice and early post-BMT, suggesting the presence of an extrathymic niche

conducive to T cell development. However, whether Notch signaling is required, as well as the cellular source(s) of Notch ligand throughout this process in the MLN, remains unknown. We hypothesize that MLNs harbor a unique environment in which Notch ligands are available to circulating progenitors and critical to sustain extrathymic T cell development in these contexts. To test this, we utilized systemic neutralizing antibodies as well as loss-of-function genetic models to assess whether the Notch ligands Dll4 or Dll1 are important for extrathymic T cell development. We found that, like in homeostatic thymocyte development, Dll4 appears to play an essential role in generation of early T cell progenitors found in the MLN. Furthermore, the source of Dll4 appears to reside within subsets of non-hematopoietic fibroblastic stromal cells lineage traced by a Ccl19-Cre transgene. In sum, these findings shed new light on the cellular and molecular cues regulating T cell development outside of the thymus.

P25. "Regulation of ACE2 expression by the microbiome"

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The COVID-19 pandemic represents a novel global health crisis. SARS-CoV-2 enters host cells upon binding to ACE2 and proteolytic cleavage by transmembrane proteases such as TMPRSS2 and TMPRSS4. In the mammalian organism, ACE2 is most highly expressed in the small intestine, and numerous clinical studies have reported gastrointestinal manifestations associated with severe COVID-19. However, the factors regulating intestinal SARS-CoV-2 infection and its importance for the overall severity of COVID-19 remain unknown.

The intestinal microbial community and the metabolites it produces play a vital role in modulating host immunity and gene expression. We made the surprising discovery that ACE2 expression is significantly increased in the absence of the microbiome, suggesting that microbial species or metabolites suppress ACE2 expression in the intestine. Using a combination of engineered diets and gnotobiotic experiments in mice, we identified microbially-produced tryptophan-derived metabolites as negative regulators of ACE2 expression in enterocytes. Many of these metabolites signal through the aryl hydrocarbon receptor (AhR), and AhR agonists reduced ACE2 expression in intestinal epithelial cells. In addition, treatment of intestinal epithelial cells with AhR agonist metabolites significantly reduced SARS-CoV-2 viral entry.

Given that tryptophan absorption in the intestine is regulated by ACE2 via the amino acid transporter B0AT1, our findings support a regulatory feedback loop between dietary nutrients, intestinal tryptophan-derived metabolites, and ACE2 expression. Collectively, these data indicate an important role for the microbiome and its metabolites in the modulation of ACE2 expression, suggesting that SARS-CoV-2 viral entry may be regulated by diet and commensal microbial colonization.

P26. "In vitro modeling of T cell exhaustion identifies novel transcriptional networks"

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Persistent stimulation of the immune system during cancer or chronic infection leads to T cell exhaustion. Exhausted CD8 T cells are characterized by decreased effector function, lower proliferative potential, increased expression of inhibitory receptors (IRs), and unique transcriptional and epigenetic profiles. Exhaustion ultimately results in a stalemate in which T cells exert limited control over but cannot fully eradicate infection while minimizing damaging immune pathology. Most data about T cell exhaustion has been generating using mouse models of chronic viral infection which have limitations; for example, in vivo mouse models have low cellular yields that prohibit the use of screening and other high-throughput exploratory platforms. In contrast, an in vitro model allows for the directed study of discrete exhaustion-associated pathways in a robustly scalable manner.

We have developed an in vitro murine model of exhaustion that recreates multiple modules of the exhaustion phenotype: high IR expression, exhaustion-associated transcription factor (TF) signature, and decreased production of effector cytokines. Furthermore, this in vitro model recreates exhaustion-specific transcriptional and epigenetic signatures, which offers novel insight into and can be combined with new and exciting technology to creatively probe the biology of exhaustion. We have performed in vitro CRISPR screening of exhaustion-related TFs to investigate the contributions of specific genetic loci to initiation and maintenance of the exhaustion program. Integration of this analysis with transcriptional and epigenetic data from RNA- and ATAC-seq analysis has identified two TF paralogues, Bhlhe40 and Bhlhe41, that are important for regulating effector- and exhaustion-specific transcriptional networks, respectively. Thus, the development and application of this in vitro model of T cell exhaustion is a useful tool for both research and development, and will allow us to mechanistically interrogate the basic biology of exhaustion while scaling up for translational discovery-oriented assays.

P27. "Role of FBXW7 isoforms in normal and neoplastic human B cells"

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Traditionally, B-cell development is thought to be orchestrated by expression of various transcription factors. However, underlying mechanisms are more complex and involve function of transcript variants. We aim to determine the extent to which transcript variants contribute to human B-cell development and neoplastic transformation. We isolated various normal human hematopoietic progenitor and B cell subsets from bone marrow and tonsils and malignant cells from primary B-Cell Acute Lymphoblastic Leukemia (B-ALL) patient samples for RNA-Seq. Data analysis with MAJIQ algorithm identified thousands of genes that give rise to transcript variants. Multiple isoforms from one gene, F-box and WD Repeat Domain Containing 7 (FBXW7), are differentially expressed not only during normal B-cell development but also in malignant samples. FBXW7 encodes an E3 ubiquitin ligase that targets substrates to proteasomal degradation. Three coding isoforms of FBXW7 -- alpha, beta, and gamma -- differ in their subcellular localization. Although others have found that FBXW7 alpha could either promote or suppress cancer in different cellular contexts, role of FBXW7 beta remains unknown in normal and malignant B cells. We found that the transition from early progenitors to pro-B is accompanied by the alpha-to-

beta isoform switch, which is reversed at later stages of normal B-cell development. B-ALL samples from Children's Hospital of Philadelphia and St. Jude Children's Hospital exhibit differential FBXW7 alpha-to-beta ratios compared to normal counterparts. Usage of FBXW7 beta exon in B-ALL patient samples is positively correlated with overall FBXW7 RNA expression. Although we detected full-length FBXW7 beta RNA transcript with Nanopore long-read sequencing and detected polyribosome binding to FBXW7 beta RNA, detection of FBXW7 beta protein remains a major challenge due to lack of suitable antibodies and short protein half-life. We overexpressed FBXW7 beta protein isoform and detected two beta-specific peptide sequences by mass spectrometry, circumventing the technical problems. Targeted proteomics approach will be developed to further address FBXW7 beta protein levels in B-ALL cells. In conclusion, expression of FBXW7 isoforms is regulated throughout human B-cell development. We hypothesize that various FBXW7 isoforms, in particular alpha and beta, target distinct substrates for degradation, which achieves temporal regulation of B-cell development at homeostasis and can play a pathological role in neoplastic transformation.

P28. "Developmental trajectory of prehematopoietic stem cell formation from endothelium"

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Hematopoietic stem and progenitor cells (HSPCs) in the bone marrow are derived from a small population of hemogenic endothelial (HE) cells located in the major arteries of the mammalian embryo. HE cells undergo an endothelial to hematopoietic cell transition, giving rise to HSPCs that accumulate in intra-arterial clusters (IAC) before colonizing the fetal liver. To examine the cell and molecular transitions between endothelial (E), HE, and IAC cells, and the heterogeneity of HSPCs within IACs, we profiled ~40 000 cells from the caudal arteries (dorsal aorta, umbilical, vitelline) of 9.5 days post coitus (dpc) to 11.5 dpc mouse embryos by single-cell RNA sequencing and single-cell assay for transposase-accessible chromatin sequencing. We identified a continuous developmental trajectory from E to HE to IAC cells, with identifiable intermediate stages. The intermediate stage most proximal to HE, which we term pre-HE, is characterized by increased accessibility of chromatin enriched for SOX, FOX, GATA, and SMAD motifs. A developmental bottleneck separates pre-HE from HE, with RUNX1 dosage regulating the efficiency of the pre-HE to HE transition. A distal candidate Runx1 enhancer exhibits high chromatin accessibility specifically in pre-HE cells at the bottleneck, but loses accessibility thereafter. Distinct developmental trajectories within IAC cells result in 2 populations of CD45+ HSPCs; an initial wave of lymphomyeloid-biased progenitors, followed by precursors of hematopoietic stem cells (pre-HSCs). This multiomics single-cell atlas significantly expands our understanding of pre-HSC ontogeny.