Presenter: Abdelfattah, Ahmed

Visualizing the Effect of osteopathic lymphatic techniques on immune system in normal subjects

Ahmed Abdelfattah,
Cairo University

Purpose: This study was designed to investigate the Effect of selected osteopathic lymphatic techniques on immune system in normal subjects. Method: Forty five subjects (33 males and 12 females), with age ranged from 20 to 30 years old participated in this study. They were assigned into three equal groups each one has 15 subjects: group A received sternal pump and sternal recoil techniques. Group B received thoracic lymphatic pump and splenic pump techniques. Group C (control group) did not receive any physical therapy modality. Absolute count of CD4 and WBCs count were used to evaluate participants before and after application of the osteopathic techniques, and for subjects in the control group before and after one month. Results: Statistical analysis revealed that there was a significant increase in CD4 P value was = 0.045 and WBCs count P-value was = 0.006 between before and after treatment with the second group in the two experimental groups. While there was no significant difference in the same measuring variables in the first and control groups. Comparison between groups revealed that there was a significant difference between the first and second groups in CD4 and WBCs, P :< 0.05.Conclusion: The second osteopathic manipulative treatment group was the effective method of enhancing the immune system in normal subjects. Key words: Osteopathy _ CD4 _ Thoracic lymphatic pump _ splenic pump technique_Sternal pump technique and Sternal recoil technique. Funding: None. Ethical committee approval: Cairo university faculty of physical Therapy: P.T.R.E/012/00945. ANZCTR NO: ACTRN12616000216415

Presenter: Abt, Michael

Host immunity modulates efficacy of microbiome transplantation in treatment of Clostridioides difficile infection

Michael Abt, Eric R. Littmann, Jung-Jin Lee, Joshua Denny, Rebecca A. Carter, Boj Susac, Isma Zarin, Kyle Bittenger

University of Pennsylvania

Clostridiodes difficile is an opportunistic pathogen that infects the large intestine following perturbation of the intestinal microbiome causing epithelial damage and debilitating, potentially fatal, colitis. Fecal microbiome transplantation (FMT) is a clinically proven therapy to treat recurrent C. difficile infection. Despite remarkable efficacy, implementation of FMT therapy is limited and the mechanism of action remains poorly understood. Here, we demonstrate a critical role for CD4+ T cells in supporting FMT success using a murine C. difficile infection system. Following FMT, chronically infected wild-type mice resolve C. difficile while cohoused, littermate Rag1-/- mice fail to clear the infection. FMT in chronically C. difficile infected mice that lack B cells, CD8+ T cells or CD4+ T cells reveals a necessary role for CD4+ T cells, but not B cells or CD8+ T cells, in resolution of C. difficile following FMT. Analysis of intestinal bacterial communities demonstrate the microbiota of FMT-responsive mice assimilates toward the composition of the FMT donor, while the non-responsive Rag1-/- and CD4+ T cell deficient mice exhibit impaired FMT engraftment. Further, FMT does not restore secondary bile acid pools in the cecum of non-responsive mice, an important metabolite associated with FMT efficacy. These data support a role for host immunity in determining efficacy of microbiota-based therapeutics.

Presenter: Adams (1), Walter

Pneumolysin-induced neutrophil transmigration and disruption of airway epithelium adherens junctions

Walter Adams (1), Shuying Xu (2), Rudra Bhowmick (3), Beth A. McCormick (4), Rodney K. Tweten (5), and John M. Leong (2). 1-San Jose State University, 2-Department of Molecular Biology and Microbiology, Tufts University, Boston, MA. 3-Oklahoma State University, Chemical Engineering, Stillwater, OK. 4-University of Massachusetts, Microbiology and Physiological Systems, Worcester, MA. 5-University of Oklahoma, Microbiology & Immunology, Oklahoma City, OK

San Jose State University

Streptococcus pneumoniae is a major cause of pneumonia, wherein infection of respiratory mucosa results in a robust influx of neutrophils and extensive tissue damage. Dissemination of S. pneumoniae across the lung epithelium into the bloodstream can lead to lethal septicemia and meningitis, however the mechanisms underlying this process have not been fully elucidated. Here we found that apical S. pneumoniae infection of polarized respiratory epithelial monolayers disrupted the organized peripheral localization of the adherens junction protein E-cadherin, likely compromising airway epithelial integrity. Analysis of a S. pneumoniae mutant deficient in the pore-forming toxin pneumolysin (PLY) revealed that neutrophil recruitment and adherens junction disruption were PLY-dependent. S. pneumoniae dissemination across polarized respiratory epithelial monolayers was promoted by PLY, and this bacterial transit was dramatically enhanced by neutrophil transepithelial migration. Infection of monolayers with mixtures of wild type and PLY-deficient S. pneumoniae demonstrated that the pro-dissemination activities of PLY functioned in trans. Ectopic production of PLY endowed the nonpathogenic Bacillus subtilis with enhanced dissemination across polarized respiratory epithelial monolayers and the ability to elicit robust neutrophil transepithelial migration. These data indicate that PLY is an important factor in promoting both neutrophil recruitment and disruption of pulmonary epithelium integrity, linked events that are crucial in promoting bacterial dissemination and disease progression during S. pneumoniae infection.

Presenter: Aldridge, Daniel

The impact of TIGIT on innate and adaptive responses during Toxoplasma gondii infection

Daniel Aldridge, Anthony Phan, Rene de Wall Maefyt, Christopher Hunter
University of Pennsylvania

Resistance to the parasite Toxoplasma gondii is mediated by NK and T cell production of IFN-γ, but failure to contract this response can lead to sever immunopathology. While cytokines, such as IL-10 and IL-27, are critical in limiting the immune response during infection, the role of inhibitory receptors in this process is unclear. The inhibitory receptor TIGIT is expressed on both NK and T cells during infection, while its ligand, CD155, is expressed on monocytes, macrophages, and dendritic cells. Furthermore, the co-stimulatory molecule CD226, which can compete with TIGIT in cis for CD155 binding, is also expressed on NK and T cells during infection. This suggests that the TIGIT signaling axis may be operational and relevant in limiting NK and T cell responses during toxoplasmosis. To investigate this, wildtype (WT) and TIGIT KO mice were infected with T. gondii. In this model, WT and TIGIT KO mice show comparable parasite burdens and similar NK and T cell responses at one week and four weeks post-infection. However, eight weeks post-infection TIGIT KO T cells show enhanced cytokine responses to the parasite. This suggests that during early stages of infection, TIGIT-independent mechanisms restrain the immune response, and a hierarchy of suppressive mechanisms may then exist during infection, with TIGIT eventually playing a role at later stages.

Presenter: Anzules, Jonathan

To Model Immune Homeostasis Under Normal and Autoimmune Conditions

Jonathan Anzules, Kristen Valentine, Genevieve Mullins, Katrina Hoyer
University of California Merced

Autoimmune disease is a phenomenon where an organism's immune system identifies parts of the body as foreign and targets it for destruction. There are several tolerance mechanisms that normally prevent self-reactive immune responses. However, this system sometimes fails and the progression of autoimmune disease is still largely unclear. It is theorized that autoimmune disease is initiated and perpetuated by a disruption to the homeostatic elements in the immune system. Understanding these homeostatic dynamics is crucial to understanding how autoimmune disease develops and is perpetuated. Cells are constantly interacting with each other, and the result of these conversations leads to increased survivability of a cell, stronger immune response, suppression, or change in activity. These conversations can be generalized and scaled up to a dynamic system of differing population interactions. Population dynamics can be represented by a system of differential equations. To further understand the homeostatic dynamics involved in autoimmunity, I propose to use mathematical modeling and experimental testing to define homeostatic constraints in healthy and autoimmune disease mice. The mathematical model will simulate the dynamics between naive CD4 T cells, activated CD4 T cells, T regulatory cells (Tregs), and the cytokine IL-2 in a developing system. I hypothesize that our model can predict whether autoimmune disease will develop when given the age and cellular population numbers.

Presenter: Apostol, April

Interferon gamma from acute Toxoplasma gondii infection during pregnancy impacts hematopoietic function in the fetus

April Apostol, Anna Beaudin, Kirk Jensen

UC Merced

During fetal development, rapidly expanding populations of stem and progenitor cells generate the foundation of the mature immune system. In adulthood, infection can drive cytokine-mediated inflammation that directly influences hematopoietic stem cell (HSC) function and differentiation, but much less is known about the fetal immune response to maternal infection during pregnancy. Here, we investigated the fetal hematopoietic response to maternal Toxoplasma gondii (T. gondii) infection. T. gondii is an intracellular parasite that elicits Type II, IFN?-mediated maternal immunity to prevent vertical transmission and promote parasite clearance. The production of excessive IFN? from T. gondii infection has dire consequences for the developing fetus, such as lowered birth weights and premature abortion, but the effects to the developing immune system and the signals that mediate these interactions has not previously been studied. Our examination of fetal hematopoiesis in response to maternal T. gondii infection reveals that fetal HSCs proliferate in response to maternal infection. Additionally, we observe significant and robust proliferation and expansion of Flk2+ multipotent progenitors (MPPs), which are known to be lymphoid-biased in adults. The expansion of presumably lymphoid-biased progenitors in response to infection during the fetal period is in direct contrast to the pervasive expansion of myeloid progenitors in response to adult infection. Additionally, we found that maternal-derived IFN-y crosses the fetal-maternal interface and may play a significant role in determining fetal HSC response to maternal infection. Ongoing experiments explore the functional outcomes of fetal HSCs following aberrant maternal inflammation to define how changes to their steady-state lineage output can influence the trajectory of the immune system across the lifespan.

Presenter: Bailis, Will

Mitochondrial metabolism biochemically uncouples T helper cell differentiation and function

Will Bailis,

Children's Hospital of Philadelphia

Activated CD4 T cells proliferate rapidly and remodel epigenetically before exiting the cell cycle and engaging their acquired effector function. CD4 T cell differentiation (proliferation and epigenetic remodeling) and terminal function are coordinately orchestrated by signal transduction and transcriptional remodeling; however, it remains unclear whether these processes are independently regulated by cellular biochemical composition. We performed a metabolism-targeted CRISPR/Cas9 screen in primary murine T cells to evaluate the full-landscape of metabolic processes required for the functional differentiation of T helper 1 (Th1) cells. We find that distinct modes of mitochondrial metabolism support Th1 cell differentiation and effector function, biochemically uncoupling these processes. We demonstrate that the TCA cycle is required for terminal Th1 cell effector function through succinate dehydrogenase (SDH; Complex II), yet the activity of SDH suppresses Th1 cell proliferation and histone acetylation. In contrast, we show that Complex I of the electron transport chain (ETC), the malate-aspartate shuttle, and citrate export from the mitochondria are required to maintain aspartate synthesis necessary for Th cell proliferation. Furthermore, we find that mitochondrial citrate export and malate-aspartate shuttle promote histone acetylation and specifically regulate the expression of genes involved in T cell activation. Combining genetic, pharmacological, and metabolomics approaches, we demonstrate that T helper cell differentiation and terminal effector function can be biochemically uncoupled. These findings support a model in which the malate-aspartate shuttle, citrate export, and Complex I supply the substrates needed for proliferation and epigenetic remodeling during early T cell activation, while Complex II consumes the substrates of these pathways, antagonizing differentiation and enforcing terminal effector function. Our data suggest that transcriptional programming works in concert with a parallel biochemical network to enforce cell state.

Presenter: Bapat, Sagar

Obesity Potentiates TH2 Immunopathology via Dysregulation of PPAR??

Sagar Bapat, Mark Ansel, Alexander Marson, Richard L. Gallo, Ronald M. Evans, Ye Zheng
University of California, San Francisco

How obesity affects immune function is not well understood. Clinically, obesity is strongly associated with severe TH2 immunopathology, though the physiological, cellular, and molecular underpinnings of this association remain obscure. Here, we demonstrate that obese mice are susceptible to severe atopic dermatitis (AD), a major manifestation of TH2 immunopathology and disease burden in humans. Mechanistically, we show that dysregulation of the nuclear hormone receptor (NHR) PPARγ (peroxisome proliferator-activated receptor gamma) in T cells is a causal link between obesity and the increased TH2 immunopathology. We find that PPARγ oversees a cellular metabolic transcriptional program that restrains nuclear gene expression of the chief TH2 priming and effector cytokine interleukin-4 (IL-4). Accordingly, thiazolidinediones (TZDs), potent PPARγ agonists, robustly protect obese mice from TH2 immunopathology. Collectively, these findings establish PPARγ as a molecular link between obesity and TH2 immune homeostasis and identify TZDs as novel therapeutic candidates for TH2 immunopathology. Fundamentally, these findings demonstrate that shifting physiologic metabolic states can shape the tone of adaptive immune responses to modulate differential disease susceptibility.

Presenter: Bass, Antonia

Elucidating IFN-[g]-induced human inflammasome responses to Legionella pneumophila

Antonia Bass, Sunny Shin

University of Pennsylvania

Host recognition of intracellular bacterial pathogens results in the formation of a multiprotein complex termed the inflammasome, leading to the recruitment and activation of inflammatory caspases. These caspases promote IL-1 family cytokine secretion and pyroptosis, an inflammatory form of cell death, which are critical for anti-bacterial defense. In mice, interferon-gamma (IFN-γ) is a potent inducer of the canonical caspase-1-dependent and noncanonical caspase-1-independent inflammasomes. In mice, a family of interferon-inducible GTPases known as guanylate binding proteins (GBPs) promote inflammasome responses against a variety of bacteria. The functions of mouse GBPs include promoting rupture of pathogen-containing vacuoles and bacteriolysis of cytosolic bacteria in order to release LPS and other pathogen-derived products into the cytosol, resulting in host recognition and inflammasome activation. In contrast to mice, which possess 11 GBPs, humans have 7 GBPs. The role of these IFN-γ-inducible genes in inflammasome activation in human macrophages is poorly understood. Here, we use Legionella pneumophila, an intracellular gram-negative bacterium, to elucidate the functions of these IFN-γ-induced genes to inflammasome responses in human macrophages. Our findings elucidate aspects of human innate immune response to gram-negative bacterial pathogens and may provide insight into developing therapeutics to prevent gram-negative sepsis.

Presenter: Bauer, Renate

IL-31 limits allergen-induced lung inflammation

Renate Bauer, Theresa Neuper, Harald Schwarz, Muamera Sarajlic, Daniel Neureiter, Helen Strandt, Patrick Suchanek, Stacey R. Dillon, Angelika Stoecklinger, Peter Hammerl, Richard Weiss, Jutta Horejs-Hoeck

Paris Lodron University of Salzburg

IL-31 is a Th2 cell-derived cytokine that is closely associated with pruritic (itching) skin inflammation. In fact, there are very promising therapeutic approaches inhibiting IL-31 signaling in people suffering from atopic dermatitis. Treatment significantly reduces pruritus or eczema area and allows patients to escape the vicious circle of itching and scratching. Recent work shows that increased IL-31 levels also correlate with asthma severity and might therefore play a role in allergic asthma or allergic rhinitis. However, the particular role of IL-31 in allergic lung inflammation is still unclear. Therefore, the main aim of this study was to investigate the contribution of IL-31 to allergen-induced lung inflammation. In order to mimic the human situation of enhanced IL-31 expression we used a murine model of IL-31 transgenic (IL-31tg) mice. We sensitized IL-31tg, IL-31 receptor knockout (IL-31RA-/-) and wildtype mice with the timothy grass (Phleum pretense) pollen allergen Phl p 5 and examined lung inflammation. Our data show that acute lung inflammation in terms of cell infiltration, cytokine secretion or mucin expression was clearly reduced in mice overexpressing IL-31 compared to wildtype or IL-31RA-/- animals. This suggests a regulatory or protective role of IL-31 in local lung inflammation.

Presenter: Billipp, Tyler

Tuft cell-derived acetylcholine regulates intestinal fluid and mucus secretion

Tyler Billipp, Kathleen DelGiorno, Jakob von Moltke
University of Washington

Tuft cells are rare chemosensory epithelial cells that initiate the intestinal Type 2 immune response to helminths and certain protists and commensal bacteria. Additional functions of tuft cells are not well understood. The intestinal epithelium maintains a barrier between the resident microbiota and the epithelium by the secretion of fluid and mucus. Secretion is regulated by acetylcholine (ACh) from enteric neurons; however, tuft cells also express the enzyme ChAT required for ACh synthesis and are uniquely positioned in the epithelium to coordinate localized fluid secretion in response to sensing of luminal signals. Here we show that the microbial metabolite succinate induces fluid secretion in the distal small intestine. The succinate-induced fluid secretion depends on tuft cell chemosensing and acetylcholine (ACh) synthesis and is independent of neuronal involvement. Succinate sensing by tuft cells may also regulate goblet cell compound exocytosis of mucus. Our findings suggest a model where intestinal tuft cells sense lumenal signals, such as the microbial metabolite succinate, and signal to neighboring enterocytes and goblet cells via ACh to induce rapid fluid and mucus secretion. Tuft cells might therefore replenish the mucus layer, coordinate the local removal of succinate-producing microbes, and regulate the microbial composition of the distal small intestine.

Presenter: Blair, Tiffany

Dendritic Cell Maturation Defines Immunological Responsiveness of Tumors to Radiation Therapy

Tiffany Blair, Shelly Bambina, Alejandro Alice, Terry Medler, Jason Baird, Gwen Kramer, Victoria Troesch, Marka Crittenden, Michael Gough

Oregon Health & Science University

Radiation therapy is capable of directing adaptive immune responses against tumors by stimulating the release of endogenous adjuvants and tumor-associated antigens. Within the tumor, conventional type 1 dendritic cells (cDC1s) are uniquely positioned to respond to these signals, uptake exogenous tumor antigens and migrate to the tumor draining lymph node (dLN) to initiate cross-priming of tumor reactive cytotoxic CD8+ T cells. Here we report that radiation therapy promotes the activation of intratumoral cDC1s in immunogenic tumors and this process fails to occur in poorly immunogenic tumors. In poorly immunogenic tumors, the adjuvant poly I:C overcomes this failure following radiation and successfully drives intratumoral cDC1 maturation, ultimately resulting in durable tumor cures. Depletion studies revealed that both cDC1s and CD8+ T cells are required for tumor regression following combination therapy. We further demonstrate that treatment with radiation and poly I:C significantly expands the proportion of proliferating CD8+ T cells in the tumor with enhanced cytolytic potential and requires T cell migration from LNs for therapeutic efficacy. Thus, we conclude that lack of endogenous adjuvant release or active suppression following radiation therapy may limit its efficacy in poorly immunogenic tumors, and co-administration of exogenous adjuvants that promote cDC1 maturation and migration can overcome this limitation to improve tumor control following radiation therapy.

Presenter: Bradshaw, Christine M.

TGF[b] neutralization improves humoral immunity to vaccination in aged mice

Christine M. Bradshaw, Jennifer L. Uhrlaub, Natalie Iannuzo, Janko Nikolich-Å½ugich
University of Arizona

Transforming growth factor β (TGFβ) is a pleiotropic cytokine involved in many cellular processes, including regulatory functions during anti-viral immune responses. Previously, we have shown in two models of age-related vulnerability, West Nile virus (WNV) and Chikungunya virus (CHIKV), that over-production of TGFβ correlates with decreased immune function. TGFβ neutralization during acute CHIKV infection decreases disease severity and improves neutralizing antibody titers. Ongoing experiments utilize RepliVAX-WN, a single cycle WNV vaccine, to elucidate the mechanism by which TGFβ blockade improves B cell responses in aged mice.

Presenter: Busch, Robert

Regulation of HLA-DR post-translational fate by proteases, vitamin D and retinoic acid

Robert Busch, Nakul Shah1, Sarah McDonald2, Islam Elkhodery1, Annica Preikschat1, Alessandra De Riva2, Steven GE Marsh3, JS Hill Gaston2, Michael J. Deery4

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Major histocompatibility complex (MHC) class II molecules, such as Human Leukocyte Antigen (HLA)-DR, present endosomal peptides to CD4+ T lymphocytes for the establishment of self tolerance and during immune responses; their allelic variation is a major determinant of acute transplant rejection and autoimmune disease risk. Key aspects of their post-translational fate remain unresolved, despite its potential relevance to the regulation of autoimmunity. Here, we report a novel role of the aspartyl protease, Cathepsin D, in the peptide loading-dependent turnover of HLA-DR proteins. Flow cytometry showed that intracellular HLA-DR molecules were rescued from degradation by treatment with the aspartyl protease inhibitor, pepstatin A (PepA), in monocyte-derived dendritic cells and in the myeloid model cell line, KG-1. Of the two lysosomal aspartyl cathepsins, cathepsin E was not detectably expressed; siRNA knockdown of cathepsin D (CatD) abrogated the PepA effect. Biochemical studies in KG-1 cells showed that PepA-mediated rescue was selective for DR conformations that were neither associated with invariant chain nor with stably-bound peptides. In vitro, recombinant CatD cleaved DR molecules after αF54, in a segment of the α-chain helix that changes conformation during peptide binding and exchange catalysed by DM. These findings suggest that CatD mediates lysosomal quality control of empty DR molecules, while sparing DR molecules associated with Ii or DM during maturation and peptide loading. We further obtained preliminary evidence that vitamin D (VitD) and all-trans retinoic acid (ATRA), two pleiotropic immune regulators, exert distinct, interacting effects on HLA-DR post-translational fate. ATRA treatment (1 μM, 72 hours) of KG-1 cells resulted in reduced total levels and inward redistribution of HLA-DR molecules, whereas VitD (10 nM) had no such effect but diminished HLA-DM levels. By targeted SILAC analysis, VitD and ATRA modulated the steady-state contribution of DRB3 gene products to total DR molecules. Moreover, DR protein expression was increased by VitD in DRB1*15-homozygous typing cell lines (HTCs), and ATRA had similar effects in DRB1*07 HTCs; both were abrogated by co-treatment with both ATRA and VitD. Analysis of other HTCs showed that these effects were not explained by known DRB1 promoter polymorphisms. While further studies are needed, these observations add to the possible mechanisms by which VitD might modify disease risk or disease course of HLA class II-linked autoimmune diseases, such as multiple sclerosis. Funded by Versus Arthritis (Senior Research Fellowship and Research Progression Award), the University of Roehampton, the MS Society (UK) (to R.B.) and the MS International Federation (McDonald Fellowship to N.S.). No competing interests.

Presenter: Cambier, CJ

Host-pathogen lipid interactions influence mycobacterial pathogenesis

CJ Cambier, Steven Banik, Joseph A. Buonomo, Carolyn Bertozzi

Stanford

Several lipids of the pathogen *Mycobacterium tuberculosis* are known to promote virulence at various stages of disease. However, the inability to probe these lipids during in vivo infection makes elucidation of their pathogenic mechanisms difficult. Using chemical extraction and reconstitution methods, we were able to define the lipid composition of the outer mycomembrane of *Mycobacterium marinum* prior to infection. Combining this approach with the synthesis of clickable, semi-synthetic lipids, we introduced a chemically tractable, biologically active variant of the virulence lipid phthiocerol dimycocerosate (PDIM) into the mycomembrane. We find that following infection of zebrafish larvae, PDIM spreads away from bacterial surfaces into the membranes of host cells. Importantly, we found that PDIM spreading into host epithelial cells prior to the arrival of macrophages to the site of infection was required for the bacteria to avoid toll-like receptor (TLR)-dependent antimicrobial immune responses. Structure-function analysis found that PDIMâ□□s biophysical properties promoting occupation of host membranes and subsequent virulence were dependent on PDIMâ□□s fatty acid tails containing methyl branches. In the absence of methyl branched lipid tails, PDIM exhibited decreased fluidity, the inability to spread into epithelial cells, and the inability to prevent TLR-dependent immune responses. Finally, we found that the concentration of cholesterol in host membranes directly correlated with the amount of PDIM spreading. Treating zebrafish with the statin atorvastatin lead to a decrease in host cholesterol, a decrease in PDIM spreading, and the fish were resistant to mycobacterial infection in a TLR-dependent manner. While it has long been appreciated that dyslipidemia promotes Tuberculosis (TB) pathogenesis, and that statins are associated with a decrease in TB incidence, our data finally suggest a potential molecular mechanism underlying these observations.

Presenter: Carozza, Jackie

Extracellular cGAMP is a cancer cell-produced immunotransmitter that promotes anti-cancer immunity

Jackie Carozza, Volker BÃ¶hnert, Khanh Nguyen, Gemini Skariah, Kelsey Shaw, Jenifer Brown, Marjan Rafat, Rie von Eyben, Edward Graves, Jeffrey Glenn, Mark Smith, Lingyin Li

Stanford University

Cancer immunotherapy targeting the adaptive immune system results in cures of previously considered terminally ill cancer patients. This remarkable achievement has excited basic researchers and clinicians alike to search for immunotherapies that can treat a higher percentage of patients and more cancers. We now know that an effective adaptive immune response to cancer depends on a robust innate immune response, and that Stimulator of Interferon Genes (STING) is the major innate immune pathway that sparks the anti-cancer immune cascade. The agonist of the STING pathway, 2â□□3â□□-cyclic GMP-AMP (cGAMP), is an intracellular second messenger synthesized in response to cytosolic DNA, which is prevalent in cancer cells. Here, we discover that cGAMP is also an extracellular signal that is regulated by its extracellular degradation enzyme ENPP1. After developing a potent ENPP1 inhibitor, we detected that cGAMP is continuously exported from cancer cell lines and can be increased by treatment with ionizing radiation. In mouse tumors, depletion of extracellular cGAMP abolished the curative effect of ionizing radiation. Together, extracellular cGAMP is an anti-cancer immunotransmitter that could be harnessed to treat cancers with low immunogenicity.

Presenter: Chiu, Honyin

The Transcriptional Cofactor Ski in Thymic Epithelial Cells Regulates Peripheral T Cell Responses

Honyin Chiu, Steve Ziegler

Benaroya Research Institute

The thymus is an important site for the establishment and maintenance of an appropriate immune response through positive and negative selection of developing T cells. The thymus contributes to thymocyte development through interactions with cortical and medullary thymic epithelial cells (TECs), termed cTECs and mTECs, respectively. Developing thymocytes also support the maturation and proliferation of mTECs by secreting transforming growth factor-beta (TGF-β) superfamily cytokines. Our lab has previously shown that deletion of TGF-β signaling in TECs enhanced negative selection and functional maturation of thymocytes, and increased the production of regulatory T cells. To investigate whether the proto-oncogene product Ski (Sloan-Kettering Institute), a negative regulator of TGF-β signaling, is associated with thymocyte development in homeostatic condition and inflammatory conditions, we generated mice that deleted Ski specifically in TECs (Foxn1CRESkifl/fl mice). We found that the mTEC population was decreased as predicted, however we did not find any differences in thymocyte development between the Foxn1CRESkifl/fl mice and the Skifl/fl control mice. Furthermore, antibody responses to NP-OVA were also unaffected. However, in an experimental autoimmune encephalitis (EAE) mouse model of multiple sclerosis, Ski deletion had a significantly greater protective effect than deletion of TGF-β. Overall, our finding suggests that Ski signaling in TECs regulates peripheral T cell self-reactivity responses.

Presenter: Coady, Alison

Deficiency in the antimicrobial peptide cathelicidin protects against systemic *Candida* infection

Alison Coady, Alison Coady, Simon DÃ¶hrmann, Josh Olson, Victor Nizet
University of California, San Diego

There is an urgent need for novel treatment strategies to combat the rising incidence of antimicrobial resistance. Indeed, the increasing emergence of antifungal resistant *Candida* species in the clinic has led the CDC to name fluconazole-resistant *Candida* as a serious threat to public health. Antimicrobial peptides (AMPs) are an attractive alternative and/or supplement to antifungals due to their ability to control fungal growth. The human AMP cathelicidin (LL-37) has been shown to display direct antifungal activity *in vitro* against the fungal pathogen *C. albicans* via membrane disruption, inhibition of adherence, and modulation of biofilm formation. However, the role of LL-37 during *in vivo* infection remains enigmatic, where LL-37 has both antimicrobial and immunomodulatory activity. For example, while previous studies have shown that mCRAMP (the murine homolog of LL-37) is dispensable for controlling *C. albicans* growth in whole blood killing assays and during subcutaneous infection, more recent work demonstrated that mCRAMP can protect against *C. albicans* colonization and dissemination in antibiotic-treated mice co-colonized with commensal bacteria. Here, in contrast, we find that loss of mCRAMP using CRAMP-deficient mice (Cramp-/-) protects against a systemic lethal infection with *C. albicans*, while wild-type mice begin to succumb to infection 24 hours post inoculation. Strikingly, there is no difference in fungal burden between the Cramp-/- mice and wild-type mice at 24 hours, suggesting that wild-type mice experience a detrimental host response, such as sepsis. Production of IL-1β, an important cytokine that protects against fungal infection, is increased in Cramp-/- mice. Preincubation of human neutrophils with LL-37 reduces neutrophil response to *C. albicans*, suggesting that LL-37 may negatively regulate phagocyte recruitment and activation. Ongoing work includes investigating the role that cathelicidin plays in modulating phagocyte recruitment and activation *in vivo*. Finally, we are exploring whether cathelicidin plays a similar role in systemic infection with other *Candida* species, including the emerging antifungal resistant *C. auris*. Ultimately, this work will not only define the normal physiological role for the LL-37 AMP during systemic candidiasis but also inform the development of AMPs for therapeutic application in patients.

Presenter: Cordova, Anthony

SLC19A1 is an importer of the immune second messenger 2â□□3â□□-cGAMP

Anthony Cordova, Christopher Ritchie, Gaelen Hess, Michael Bassik, Lingyin Li

Stanford University School of Medicine

Analogs of the innate immune second messenger 2â□□3â□□-cGAMP have potent antitumoral effects in mice, and several of these analogs are now in clinical trials for metastatic solid tumors. 2â□□3â□□-cGAMP and other cyclic dinucleotides (CDNs) bind to the ER-membrane protein STING, leading to transcription of type I interferons and downstream immune activation. However, it is unknown how extracellular 2â□□3â□□-cGAMP and its analogs cross the cell membrane to activate intracellular STING. Using a genome-wide CRISPR screen we identified SLC19A1 as the first known importer of cGAMP and other CDNs, including the investigational new drug 2'3'-bisphosphosphothioate-cyclic-di-AMP (2'3'-CDAS). These discoveries will provide insight into cGAMP's role as an immunotransmitter, and will aid in the development of better therapeutic STING agonists with greater target cell selectivity, fewer off-target effects, and better patient stratification.

Presenter: Covarrubias, Sergio

CRISPR screening in macrophages identifies membrane-bound TNF as an autocrine-acting negative regulator of inflammation

Sergio Covarrubias, Apple Vollmers, Allyson Capili, Michael Boettcher, Elektra K. Robinson, Laura Oâ□□Briain, Christopher Vollmers, James Blau, Michael McManus, Susan Carpenter

UCSC

Excess inflammation is associated with a variety of autoimmune diseases and cancers. Macrophages are critical cells of the innate immune system involved in the recognition and destruction of invading microbes in addition to the resolution of inflammation. Understanding the entire catalog of genes involved in the inflammatory response is essential to gaining new insights into immune dysregulation that occurs in autoinflammatory diseases. NF-κB is a major transcription factor that drives the inflammatory response. Here we utilize an NF-κB reporter macrophage line to perform a fluorescence-activated cell sorting (FACS)-based whole-genome CRISPR screen. We identify 115 novel positive and negative regulators of the NF-κB pathway. Unexpectedly, we identify the pro-inflammatory cytokine, TNF, as negative regulator of inflammation and confirm that it acts in an autocrine-manner likely through binding the inhibitory p75 (Tnfrsf1b) receptor. In summary, our NF-κB-CRISPR screen uncovers novel regulators of inflammation and reveals important regulatory complexities of the TNF pathway.

Presenter: Cowan, Courtney

Local Delivery of IL-12 via Genetically Engineered Macrophages Activates Adaptive Immunity

Courtney Cowan, Katherine J Brempelis, Kevin P Labadie, Shannon A Kreuser, Harrison K Chinn, Amira Davis, Venu G Pillarisetty, Courtney A Crane

Seattle Children's Research Institute

Eradication of solid tumors is challenging, particularly as many tumors are hard to target due to low antigenic burden and their immunosuppressive tumor microenvironment (TME). Additionally, one-time local deliveries of treatment often fail to create a robust, lasting immune response, and multiple local deliveries are typically accompanied by adverse side effects and arenâ□□t always feasible. While some therapies, such as the pro-inflammatory cytokine interleukin-12 (IL-12), exhibited promising pre-clinical results, clinical trials revealed that systemic delivery of the cytokine results in toxicity. To combat the challenge of systemic toxicity, we lentivirally transduced macrophages to achieve constitutive expression of IL-12. We hypothesized that these genetically engineered macrophages (GEMs) would allow for local, sustained, and low-dose production of IL-12 that would reinvigorate the adaptive immune response within the immunosuppressive TME. In vitro, we demonstrate that the IL-12 produced by GEMs is titratable and sustained for 30 days post transduction. T-cells cocultured with IL-12 GEMs show increased activation, proliferation, and interferon-gamma production. When cocultured with human tumor tissue slices, IL-12 GEMs caused more tumor death than the control GEMs or IL-12 alone. While this platform is a promising local delivery option for cancer treatments, it also has the versatility for applications in other areas of medical science, such as autoimmune disease.

Presenter: Cribas, Emily

IL-10 deficiency promotes microbiota-driven IL-22 mediated defenses against acute C. difficile infection

Emily Cribas, Emily S. Cribas, Jeffrey Maslanka, Joshua Denny, Michael C. Abt
University of Pennsylvania

Clostridium difficile infection is the most common hospital-acquired infection in the United States. Pathogenesis is initiated by toxin-mediated damage to the intestinal epithelium and is exacerbated by non-C.difficile bacterial translocation. While the contributions of commensal bacteria and host immune responses in shaping C.difficile pathogenesis have been well-studied, the indirect role of commensal bacteria-mediated immunoregulation on disease progression remains poorly understood. Given the importance of the anti-inflammatory cytokine IL-10 in microbial tolerance, we use Il10-/- mice as a model to study dysregulated proinflammatory responses to commensal bacteria in the context of C. difficile infection. We show that specific pathogen-free Il10-/- mice have reduced morbidity and mortality to acute C. difficile infection compared to littermate Il10 heterozygous (Il10HET) mice. Improved survival was independent of C.difficile burden and toxin levels at peak infection, suggesting a critical role for immune defenses against non-C.difficile bacteria. Although the immune response at the peak of infection did not differ significantly between Il10-/- and Il10HET mice, there was elevated baseline expression of il22 and ifng in Il10-/- mice; both cytokines are critical in mediating innate immune defense against C.difficile. Genetic ablation of T-bet, the master transcriptional regulator of IFN-? production, in Il10-/- mice, did not diminish protection against severe C. difficile infection. In contrast, il10.il22 double knockout mice were significantly more susceptible compared to il10-/- mice. We conclude that absence of IL-10 improves host defense against C.difficile pathogenesis in an IL-22 dependent manner. Future work will involve defining the mechanism of IL-22 induction and identifying downstream effectors required for protection upon loss of IL-10 and commensal tolerance.

Presenter: Crowl, Ty

Defining the transcriptional adaptations of tissue-resident CD8+ T cells to diverse non-lymphoid tissues

Ty Crowl, Clara Toma, Amir Ferry, Kyla Omilusik, Ananda Goldrath

University of California - San Diego

CD8+ T cells are critical components of effective immune responses to intracellular pathogens and tumors. Upon recognition of infection, CD8+ T cells dramatically expand in number, drive inflammation, and kill infected cells. After infection is cleared, a small subset of long-lived, pathogen-specific CD8+ T cells remain as memory cells, providing long-term protection against previously encountered pathogens. Recently, it has been appreciated that some memory CD8+ T cells remain lodged in tissues, acting as sentinels and guarding common sites of pathogen entry. Over the last several years, many of the mechanisms by which tissue-resident memory cells (TRM) are generated and maintained have been revealed. However, TRM in diverse tissues encounter a range of microenvironments that vary widely in terms of nutrient availability, oxygen tension, pH, and cytokine milieu. The contributions of diverse tissue microenvironments to TRM differentiation are unknown, yet are likely essential for the generation of protective, localized memory responses. We have used RNA-seq, ATAC-seq, and single cell RNA-seq to understand the diversity of TRM populations in distinct tissue microenvironments and identify genes critical for tissue-specific TRM differentiation. We sequenced circulating CD8+ T cells from the spleen and blood and TRM from the small intestine, kidney, salivary gland, fat, and liver. Using these data, we identified multiple tissue-specific transcriptional programs of TRM, and we found that the transcriptional repressor Hic1 is a critical regulator of small intestine-specific TRM differentiation. Elucidating how TRM populations adapt to distinct microenvironments and the tissue-specific transcriptional regulators that mediate these changes will be critical for enhancing protective memory responses at the sites most vulnerable to infection.

Presenter: D'Souza, Lucas J

2NBDG uptake is distinct from glucose transport and contributes to plasma cell longevity

Lucas J D'Souza, Wing Y Lam, Stephen H Wright, and Deepta Bhattacharya

Department of Immunobiology, University of Arizona College of Medicine

Plasma cells are terminally differentiated B lymphocytes whose dominant function is antibody production. These antibodies are largely protective in nature, and the durability of the humoral response against invading pathogens correlate with the lifespan of the plasma cell. However, little is known about mechanisms contributing to the long life of these cells. We have previously demonstrated that long-lived plasma cells possess a higher mitochondrial spare respiratory capacity than their short-lived counterparts, thus offering a metabolic basis for plasma cell longevity. This property depends on the on the cellsâ□□ ability to import glucose, as measured by uptake of the fluorescent glucose analog, 2NBDG. Counterintuitively, we found that expression of the glucose transporter Slc2a1 to be similar between ex vivo 2NBDG+ and 2NBDG- plasma cells. Further, genetic ablation of Slc2a1 in a myeloma line using CRISPR-Cas9 did not affect 2NBDG uptake and its kinetics despite reduced 14C-glucose uptake. Put together, our findings suggest that 2NBDG and glucose transport in plasma cells are mediated through different transporters. In addition to Slc2a1 and lesser expressed sugar transporters, abrogation of nucleoside and nucleotide-sugar transporters in a myeloma line had no effect on 2NBDG uptake; thus, ruling out a possible mimicry of naturally occurring biomolecule/s. In conclusion, as 2NBDG unequivocally marks long-lived plasma cells in vivo, identifying the transporter and pathways that mediate its uptake will be of interest in dissecting mechanisms involved in plasma cell longevity.

Presenter: Davalos, Oscar

Transcriptional profiling defines unique gene expression signatures in CD8 T follicular cells

Oscar Davalos, Genevieve Mullins, Kristen M. Valentine, Katrina K. Hoyer

Department of Molecular and Cell Biology, University of California Merced

Cytotoxic CD8 T cells have been largely overlooked when defining autoimmune pathology. A population of CXCR5+ CD8 T cells arises during autoimmune disease. This research aims to identify the similarities and differences in CXCR5+ CD8 T cells relative to CD4 T follicular helpers and bulk activated CD8 T cells using the IL-2-KO autoimmune model. RNA-seq was performed on CXCR5+PD-1+ CD8 T cells and CD4 T follicular helper cells from IL-2 deficient mice at 15 days of age. For transcriptional profiling, CXCR5+ CD8 T cells were compared to the average of CD4 T follicular helper and bulk CD8 T cell expression. We identified 2030 differentially expressed genes, with 1157 upregulated and 873 downregulated. CXCR5+ CD8 T cells retained cytotoxic gene expression and expressed a subset of genes specific to this population. Top 15 significant gene ontology categories indicated immune responses and cellular movement. We are currently evaluating the functionality of the genes found specifically in CXCR5+ CD8 T cells, and exploring the transcriptional differences in CXCR5+ T cells based on autoimmune disease pathology.

Presenter: Deets, Katie

Investigating a role for the NAIP/NLCR4 inflammasome in adaptive T cell responses

Katie Deets, Jakob Von Moltke, Russell Vance

UC Berkeley

Activation of the NAIP/NLCR4 inflammasome aids in the elimination of certain cytosolic bacteria from the intestinal epithelium. NAIP/NLCR4 activation in these cells results in pyroptosis and expulsion of the infected cell into the lumen of the gut. However, it remains unclear what effect this rapid cell expulsion has on pathogen-specific CD8 T cell responses. To address the role of the NAIP/NLCR4 inflammasome activation and cell expulsion on a subsequent CD8 T cell response, we are using a novel in vivo genetic system that uses a tamoxifen-inducible Cre recombinase to express flagellin (Fla), a potent activator of NAIP/NLRC4, along with the model antigen ovalbumin (Ova) as a single fusion protein (OvaFla) in the cytosol of intestinal epithelial cells (IECs). This tool allows us to address several key questions: (1) Are antigens from pyroptotic IECs released into the underlying intestinal tissue? (2) Are these antigens taken up and cross-presented to CD8 T cells? (3) Is this cross-priming response sufficient to generate protection against future infections? We will share our ongoing results addressing these questions.

Presenter: Diaz-Ochoa, Vladimir

NRAMP1 Is Critical for Neutrophil-Mediated Control of Intracellular Pathogens

Vladimir Diaz-Ochoa, Kristen L. Lokken, Ariel MuÃ±oz, Annica R. Stull-Lane, Jason P. Mooney, and Renee M. Tsolis

University of California, Davis

The Natural Resistance Associated Macrophage Protein 1 (NRAMP1) is a prominent metal transporter in host defenses against pathogens. Prevailing scholarship holds that macrophages facilitate control of intracellular pathogens via NRAMP1 mediated metal starvation in the phagosome. We found that NRAMP1 in neutrophils also contributes to host control of bacterial pathogens. While investigating how vitamin A deficiency impairs immunity against systemic salmonellosis, we discovered that NRAMP1-deficient animals were equally susceptible to disseminated *Salmonella* as vitamin A-deficient, NRAMP1-proficient, mice. This data suggested that NRAMP1 mediated immunity to disseminated salmonellosis was dependent on vitamin A. Indeed, Vitamin A deficiency impaired infection-induced granulopoiesis, resulting in reduced expression of specific- and gelatinase granule components in neutrophils, including NRAMP1. Adoptive transfer of neutrophils from NRAMP1-proficient donors, but not NRAMP1-deficient donors, reduced the systemic *Salmonella* burden in vitamin A-deficient, NRAMP1-proficient, mice and in NRAMP1-deficient animals. Additionally, NRAMP1-deficient neutrophils displayed diminished killing of *Salmonella ex vivo* compared to NRAMP1-proficient neutrophils. During infection with another vacuolar intracellular pathogen, *Brucella abortus*, we found that NRAMP1 contributed to a reduction in *Brucella* burden of the placenta, in which neutrophils also play a prominent role. Collectively, these data suggest that NRAMP1-deficiency impairs control of intracellular pathogens by blunting neutrophil-mediated host defenses.

Presenter: Diehl, Gretchen

Early life selection of gut microbiota specific T cells

Gretchen Diehl, Daniel F. Zegarra-Ruiz, Dasom Kim, Kendra Norwood, Shubhabrata Majumdar, Wan-Jung Wu, Myunghoo Kim, Matthew Bettini

Baylor College of Medicine

AIRE-dependent expression of tissue-specific antigens by medullary thymic epithelial cells allows for the selection of non-autoreactive T cells, supporting self-tolerance. Although gut microbiota is known to educate the immune system in the periphery, balancing effector and regulatory T cells responses, it remains unknown how bacteria-specific T cells are selected. Here we studied how thymic T cell development is altered after colonization with model intestinal microbes. Colonization of mice at weaning induced thymic expansion of bacteria-specific naive T cells. Remarkably, bacterial DNA was found in the thymus of colonized mice and 16s rDNA sequencing identified bacterial DNA from a broad range of intestinal microbes in the thymus of steady-state mice. We identified enrichment of CX3CR1+ dendritic cells (CX3+ DCs) after microbial colonization, and depleting these cells or inhibiting their antigen-presentation suppressed induction of bacteria-specific T cells. Further, depletion of the gut microbiota with antibiotics reduced positive selection, bacterial 16s, and CX3+ DCs in the thymus. In older mice, we did not observe microbial DNA in the thymus, altered CX3+ DCs, or expansion of microbiota specific T cells, indicating a developmental window where intestinal microbes can regulate thymic output. Using models of microbiota-specific T cell immunity or pathogen infection, we demonstrate thymic microbiota-specific T cells can differentiate into intestinal effector T cells, offering protection or mediating pathology. Together, our data suggests trafficking of bacteria to the thymus by intestinal DCs drives T cell selection of microbiota-specific T cells to shape peripheral immunity against pathogens and the microbiota itself.

Presenter: Diep, Anh

Innate immune cell influence on adaptive immune cell function during Coccidioides infection

Anh Diep, Kelly Otsuka, Katrina H. Hoyer
University of California, Merced

Coccidioidomycosis is a fungal, respiratory disease caused by Coccidioides immitis and Coccidioides posadasii. This emerging infectious disease ranges from asymptomatic to pulmonary disease with disseminated infection. There is little understanding as to what differentiates hosts that resolve the disease and hosts that development chronic infection. Intervention at early disease stages is difficult as symptoms presented are broad and generic. There remains a critical need for understanding coccidioidomycosis and identifying biomarkers of disease progression for more effective diagnosis and treatment. Coccidioides infection occurs when arthroconidia is inhaled into the lung and undergoes morphological changes from soil (arthroconidia) to host form (spherule). The initial immune response is mediated by local cells within the lung epithelium tissue and recruited immune cells. Effective Coccidioides clearance requires monocyte migration into the site of infection and subsequent differentiation. Macrophages and neutrophils mediate fungal clearance via phagocytosis and effector cytokine secretion. Long lasting immunity to Coccidioides requires dendritic cell activation of effector helper cells. Little is known about the dynamics and interactions between immune cells and Coccidioides at the start of infection and how these interactions influence later adaptive immune cell recruitment and function. Our lab observed a striking difference between chronic disease patients and patients who cleared. These differences in immune cell profiles and cytokines in serum lead us to investigate innate immune cell response to Coccidioides and their influences on adaptive immunity in vitro and in vivo.

Presenter: DiRenzo, Daniel

Dual A2aR/A2bR antagonism with AB928 suppresses the effects of adenosine on both immune and cancer cells in the tumor microenvironment

Daniel DiRenzo, Kelsey ES Gauthier, Sean Cho, Akshata Udyavar, Sachie Marubayashi, Kristen Zhang, Adam Park, Dana Piovesan, Jesus Banuelos, Devika Ashok, Jenna L Jeffrey, Lisa Seitz, Manmohan R Leleti, Jay P Powers, Matthew J Walters

Arcus Biosciences, inc.

There are several mechanisms of immune suppression in the tumor microenvironment (TME). In addition to engagement of immune checkpoint receptors commonly found on T cells in the TME, high levels of extracellular adenosine also inhibit anti-tumor responses through activation of the A2aR and A2bR adenosine receptors. Expression of A2aR and A2bR can vary by cell type, and the contribution of A2bR to the suppressive TME is not well understood. We have previously shown that AB928, a dual A2aR/A2bR antagonist, blocks the immunosuppressive effects of adenosine in cultured human immune cells and mouse syngeneic tumors. Using dual and selective adenosine receptor antagonists, here we assessed the contribution of A2bR signaling to adenosine-mediated immunosuppression. We and others have shown that T cells and other non-myeloid cells predominantly express A2aR, whereas myeloid cells, such as dendritic cells (DCs), express both A2aR and A2bR. Experiments in vitro showed that human CD14+ monocyte-derived DCs (moDCs) have the highest levels of A2bR expression compared to cultured monocytes and macrophages. Notably, moDC differentiated in the presence of adenosine showed a decreased ability to stimulate IFN-? secretion from allogeneic CD4+ T cells. AB928 was able to attenuate the suppression of IFN-? by adenosine and showed significantly greater rescue than a comparable A2aR-only specific antagonist. NanoString gene expression profiling identified 87 genes (>2.0 fold change, p

Presenter: Dulson, Sarah J.

Nrf2 Protects Against Immune Dysfunction Following Burn Injury

Sarah J. Dulson, Tim Eitas, Lucas Sjeklocha, Michelle Mac, Shannon Wallet, Bruce Cairns and Robert Maile

University of North Carolina at Chapel Hill

Patients with severe burn injury experience an acute systemic immune dysfunction. This results in increased susceptibility to potentially devastating hospital-acquired infections. Additionally, pulmonary stress can originate from systemic inflammation in burn patients and is compounded by inhalation injury. The transcription factor Nuclear Factor-Erythroid-2-Related-Factor (Nrf2) activates downstream of innate sensing receptors and engages an antioxidant and detoxifying gene program that serves to limit immunopathology. We find that Nrf2-deficient (Nrf2-/-) mice are unable to control immune responses following burn and burn + inhalation (B+I) injury, demonstrated by increased edema, vascular permeability, and mortality. Furthermore, significantly higher concentrations of circulating cytokines, including IL-6, TNFα, and MCP-1, were measured in Nrf2-/- animals following B+I injury. Interestingly, lung tissue from wildtype mice show increased expression of Nrf2 after injury, but analysis of its cellular localization revealed that most Nrf2 was confined to the cytoplasm and, thus, unable to direct gene transcription. Therefore, we conclude that Nrf2 is required following B+I injury but insufficiently activated. We propose that therapeutic enhancement of the Nrf2 pathway is a novel method to counteract immune dysregulation and could lead to improved patient outcomes following burn injury.

Presenter: Elliott, Jennifer

Interaction between HIV-1 integrase and viral RNA drives proper virion morphogenesis and is necessary for successful infection

Jennifer Elliott, Jenna E. Eschbach, Pratibha C. Koneru, Wen Li, Maritza Puray-Chavez, Dana Townsend, Dana Lawson, Alan N. Engelman, Mamuka Kvaratskhelia, Sebla B. Kutluay

Washington University School of Medicine

Recognition of non-self RNA or DNA is a critical step in the immune response to many viral pathogens. Viruses in turn have evolved multiple means of evading or subverting sensing mechanisms, with a common strategy being limiting the accessibility of viral nucleic acids. In the case of HIV-1, the viral genomic RNA is enclosed inside a conical capsid lattice, which is released into the target cell upon viral entry. In addition to its structural role, the HIV-1 capsid is thought to be essential for shielding the viral genomic RNA from cytosolic pattern recognition receptors. Recent findings indicate a key role for the HIV-1 integrase (IN) enzyme in proper virion morphogenesis, and mutations within IN can lead to the generation of morphologically aberrant viral particles with the viral genomic RNA mislocalized outside the capsid core. Despite containing all the components necessary for replication, such particles cannot successfully infect target cells. To determine how multiple mutations within IN can lead to similar defects in virion morphology and viral replication, we characterized a panel of IN mutant viruses for defects in the viral lifecycle. Human primary CD4+ T cells or cultured cells were infected with IN mutant viruses and viral RNA, DNA, and proteins were analyzed by either immunoblot, qPCR, or confocal microscopy. All of the mutant viruses screened were severely attenuated in infectivity, and encountered a block early after entering target cells. Viral RNA and IN itself were prematurely lost from target cells, and reverse-transcribed viral DNA did not accumulate. Similar defects were seen in viruses in which the capsid protein (CA) was mutated to destabilize the capsid core. These findings support a model in which the HIV-1 capsid core is critical for protecting entering viral RNA and replicative enzymes from the host environment.

Presenter: Emami, Michael

Characterizing the immune response to AAV9-CRISPR/Cas9 in a humanized mouse model of Duchenne muscular dystrophy using single cell RNA-sequencing

Michael Emami, Michael R Emami, Courtney Young, Feiyang Ma, Matteo Pellegrini, Melissa Spencer

University of California, Los Angeles (UCLA)

Duchenne muscular dystrophy (DMD) is an x-linked disease caused by out-of-frame mutations in the DMD gene, which encodes dystrophin. Due to the nature of these mutations, DMD is amenable to gene-editing and gene replacement therapies. Ongoing phase I/II clinical trials for DMD rely on a gene replacement strategy using adeno-associated virus (AAV) to achieve systemic delivery. However, immune responses against AAV vectors have resulted in serious adverse events (SAEs) in current and past studies. Yet, it is poorly understood why certain patients develop SAEs following AAV gene therapy and what specific immune responses arise. To study immune responses in vivo following AAV administration, we dosed a humanized dystrophic mouse model (hDMD del45) with AAV serotype 9 carrying CRISPR/Cas9 and performed 10x Genomics single cell RNA-sequencing (scRNA-seq). We tested three different AAV9-CRISPR/Cas9 doses: 1.2E12vg, 6E12vg, and 1E13vg and isolated PBMCs pre-AAV administration and 4 weeks post-AAV administration. Four main immune cell populations were observed: monocytes, NK cells, B cells, and T cells, all of which demonstrated a clear shift in phenotype between pre- and post-AAV treatment. Within the T cell population, we detected shifts in the phenotypes of all subpopulations including CD4+, CD8+, regulatory T cells (Tregs), and gamma delta (?d)T cells between pre- and post-AAV treatment, regardless of dose. Assessment of gene expression within T cell subpopulations revealed upregulation of Dusp1 and Dusp2 in CD8+, CD4+ and ?dT cells. These two genes are downstream of Toll-Like Receptor signaling and may represent a potential target to suppress SAEs with AAV administration. Additional analysis and validation studies are needed identify critical immune cell populations and genes that elicit responses to AAV, and to separate capsid vs transgene-specific immune reactions. These studies will enable the identification of new target genes involved in immune responses to AAV.

Presenter: Ergun, Sabrina L.

Interrogating the Molecular Mechanisms of STING Signaling

Sabrina L. Ergun, Daniel Fernandez, Thomas M. Weiss, and Lingyin Li
Stanford University

The STING (STimulator of INterferon Genes) pathway is an innate immune signaling cascade which promotes essential anti-cancer, anti-viral, and anti-bacterial responses. Conversely, STING overactivation is linked to several autoimmune and inflammatory diseases such as Lupus, Multiple Sclerosis, heart attack, and Parkinsonâ□□s disease. Despite its significance in the disease context, the precise molecular mechanism of STING activation and attenuation remains unclear. Using structural biology and biochemistry, we report that the metazoan second messenger 2â□□3â□□-cGAMP induces the release of the auto-inhibiting STING C-terminal tail, which exposes a polymerization interface on the STING dimer and leads to the formation of disulfide-linked polymers via cysteine residue 148. Autoimmune disease-causing hyperactive STING mutations either flank C148 and depend on disulfide formation or reside in the putative C-terminal tail binding site and cause constitutive C-terminal tail release and polymerization. Finally, bacterial cyclic-di-GMP induces an alternative active STING conformation, activates STING in a cooperative manner, and acts as a partial antagonist of 2â□□3â□□-cGAMP signaling. Our insights explain the tight control of STING signaling given varying background activation signals and provide a novel therapeutic hypothesis for autoimmune syndrome treatment.

Presenter: Forero, Adriana

Type I Interferon-mediated Activation of IRF1 Underlies the Distinct Inflammatory Responses Elicited by Type I and Type III Interferons

Adriana Forero, Snehal Ozarkar, Hongchuan Li, Chia H Lee, Emily A Hemann, Marija S Nadjsombati, Matthew R Hendricks, Lomon So, Richard Green, Chandra N Roy, Saumendra N Sarkar, Jakob von Moltke, Stephen K Anderson, Michael Gale Jr, Ram Savan

University of Washington, Department of Immunology

The epithelium is a highly proliferative tissue under constant exposure to pathogenic and commensal microbes. There type I (α/β) and III interferons (λ) (IFNs) activate similar downstream signaling cascades that result in kinetic differences in the expression of antiviral IFN-stimulated genes (ISG). We address the fundamental question of why these two IFN families are required to prevent viral dissemination at barrier sites by providing some of the molecular mechanisms underlying the disparity in their ability to induce inflammation. IFNα/β singularly induce expression of the transcription factor IRF1, with little to no IRF1 induction observed after IFN? treatment. Through genome-wide expression analysis, we demonstrate that while IRF1 expression is dispensable for the antiviral activity of IFNα/β, it is necessary for the induction of inflammatory chemokines and immune cell infiltration. The muted induction of IRF1 by IFNλ is due to insufficient STAT1 activation and homodimerization given the limited IFNλ receptor 1 subunit (IFNLR1) expression which is refractory to inflammatory stimuli or viral challenge. However, exogenous overexpression of IFNLR1 can enhance both antiviral and chemokine gene expression through the induction of IRF1 demonstrating how IFNLR1 expression regulation is crucial for preventing deleterious inflammation. On the other hand, our gene expression profiling identified genes that were expressed uniquely in IFNλ stimulated cells. Gene set enrichment and regulatory analyses suggested that many of these genes are involved in pathways consistent with the resolution of inflammation and the maintenance of barrier function. Overall, we show that IFNα/β and IFNλ work in concert to sustain antiviral immunity and limit tissue damage at sites of pathogen exposure. The transient inflammatory responses to IFNα/β help recruit immune effectors to promote protective immunity while IFN? act as potent antiviral effectors and restricting tissue damage.

Presenter: Freedman, Alicia

Hide or Die: The Alternative Fates of Autoreactive T cells

Alicia Freedman, Alicia Freedman, Stefan Abreo, Nicholas Fong, Yitzhar Goretsky, Tyler-Marie Deveau, Alisa Sas, Shahar Dubiner, and Martha C. ZÃºÃ±iga

University of California, Santa Cruz

Negative selection of developing thymocytes is the major mechanism for developing immune tolerance to self. However, not all autoreactive thymocytes are deleted. Additional thymic mechanisms (including the development of regulatory T cells) also enforce self-tolerance. Interestingly, in some transgenic mouse models in which the autoreactive TCR is specific for class I MHC, there is an elevated number of CD4-CD8- (DN) thymocytes, in addition to deletion of CD8+ thymocytes. These DN thymocytes include regular immature thymocytes and also thymocytes that can develop into NK1.1+ T cells. One such transgenic model is that of the BM3.3 TCR made in CBA (H-2k haplotype) mice. The BM3.3 TCR has high affinity for the alloantigen, H-2Kb. H-2Kb can induce signaling in naÃ¯ve BM3.3 T cells in a CD8-independent manner. In double transgenic mice in which H-2Kb expression is driven by the guinea pig ?-lactalbumin promoter (KALxBM3 mice) CD8+ thymocytes fail to develop. The thymii of these mice have an exceptionally large DN population, some of which are TCRloCD3loNK1.1+. We found a significant (~23%) population of DN thymocytes that are TCRhiCD3hi. Given that the BM3.3 TCR can signal in a CD8-independent manner, we hypothesized that these thymocytes may arise upon antigen encounter to become true DN T cells that have distinct functions. In this study, we used multi-color flow cytometry to examine the molecular signatures of the DN thymocytes in BM3, KALxBM3 (rag-proficient), and KALxBM3rag-/- (rag-deficient) mice. We are particularly interested in CD5 because an increase in CD5 expression is commonly regarded as evidence of the interaction of a thymocyte with its cognate antigen. In BM3 mice all DN thymocytes are CD3loCD5loCD62Lhi. In contrast, DN thymocytes in KALxBM3 and KALxBM3rag-/- mice occur in two populations â CD3loCD5loCD62Lhi and CD3hiCD5intCD62Lint. The latter phenotype is virtually identical to that of CD8 SP thymocytes in BM3 mice. These âantigen-experiencedâ DN thymocytes also express higher levels of the H-2Kb-specific TCR (detected with the clonotypic Ti98 antibody). A high proportion of these antigen-experienced DN thymocytes express CCR7, suggesting that they migrate to the medulla. TCRhiCD3hiDN T cells also are found in lymph nodes. A high proportion of these TCRhiCD3hiDN T cells have a memory phenotype and intermediate levels of CD5. Future studies will focus on determining the cytokine profiles and immunological function of these TCRhiCD3hiDN T cells.

Presenter: Freedman, Bruce

BCR-induced calcium signals dynamically tune key checkpoints that control the survival, metabolic reprogramming, and proliferation of naiÂ¨ve B cells

Bruce Freedman, Corbett T. Berry, Xiaohong Liu, Satabdi Nandi, Youhai Chen, Uri Hershberg, Igor Brodksy, Michael P. Cancro, Christopher J. Lengner, Michael J. May, Bruce D. Freedman

University of Pennsylvania

Functional humoral immunity arises through an antigen driven process that generates a repertoire of B cells whose antigen receptors have â□□appropriateâ□□ affinity for pathogenic antigens This is possible because the affinity and specificity of antigen and B cell receptor (BCR) interactions are encoded in the quantitative properties of intracellular signals triggered by these interactions. Mechanisms that decode these signals then determine if a cell is deleted or is rescued from apoptosis and goes on to proliferate and differentiate. Additional costimulatory and co-activating signals also have a critical impact on a cellâ□□s fate and serve to validate the authenticity of an antigen. Remarkably, the nature and regulation of these fate driving intracellular signals remains largely unresolved. We and others have established that changes in intracellular calcium concentration triggered by antigen/BCR engagement, regulate a multitude of critical subcellular processes and tune the expression of fate specific genes. However, the underlying molecular mechanisms by which calcium fine tunes these specific responses are yet to be resolved. Thus, we sought to dissect and delineate the mechanisms by which BCR induced calcium signals regulate mature B cell survival and proliferation. First, we establish that BCR-induced calcium signals rescue naÃ¯ve B cells from apoptosis through control of canonical NF-kB activation. We also demonstrate that these signals then drive a cells subsequent entry into the cell cycle through control of c-Rel and mTORC1 and that calcium dependent regulation of Myc transcription and translation promotes maximal B cell proliferation. Furthermore, we demonstrate how costimulatory signals cooperate with the BCR to fine tune a cellâ□□s fate. Altogether, our findings provide a mechanistic framework for understanding how distinct patterns of calcium signaling, generated by differences in the affinity of antigen binding to the BCR and how costimulatory signals cooperate with the BCR to fine tune a cells fate and immune function. These mechanisms are significant because each represent a potential therapeutic target that may be useful for regulating pathophysiological immune dysfunction or enhancing insufficient immune responses.

Presenter: Freeman, Brian

Saponin-MPLA adjuvant promotes robust humoral immunity by enhancing B cell antigen acquisition, TFH function, and germinal center development

Brian Freeman, Yu Kato, Murillo Silva, Hannah Watkins, Jason Chang, Shane Crotty, and Darrell J. Irvine

Division of Vaccine Discovery, La Jolla Institute for Immunology

Adjuvants are a key component of subunit vaccines that enhance the immune response. We developed a self-assembled saponin nanoparticle adjuvant containing monophosphoryl lipid A (SMNP). SMNP was superior to multiple existing adjuvants at enhancing humoral responses in mice and non-human primates. Mechanistic studies revealed that, unlike other adjuvants, SMNP rapidly increased the rate of lymphatic flow. SMNP also disrupted subcapsular sinus macrophages, thereby increasing follicular access of antigens. These actions of SMNP led to enhanced antigen uptake by B cells and other antigen presenting cells in lymph nodes, leading to efficient activation and proliferation of antigen-specific B cells and CD4+ T cells. CD4+ T cells activated in the presence of SMNP efficiently differentiated to IL-21+ T follicular helper cells (TFH), supporting potent germinal center B cell and plasmablast responses. Notably, enhanced B cell and TFH responses were observed not only in the primary draining lymph nodes but also in distal secondary lymphoid organs, consistent with the enhanced lymphatic flow and disruption of subcapsular sinus macrophages caused by SMNP. These studies indicate that SMNP is a promising new adjuvant that should be considered for clinical use to elicit consistent and robust humoral responses to candidate vaccines.

Presenter: Gallant, Robert

Hypothalamic Cooperative Defenses during Sepsis

Robert Gallant, Karina Sanchez, Jose Puerta, Janelle Ayres
Salk Institute for Biological Studies

Host defense against infections utilizes two distinct strategies. Resistance mechanisms function to promote health by decreasing pathogen burden while cooperative defenses promote health with a neutral to positive impact on pathogen burden. We sought to identify novel cooperative defenses during sepsis by utilizing the concept of lethal dose 50 (LD50). We developed an LD50 polymicrobial sepsis model in which mice are infected with a 1:1 mixture of E. coli O21:H+ and S. aureus by intraperitoneal injection. Within 8-10 hours, half of the infected mice succumb to infection while half become asymptomatic carriers with no difference in pathogen burden. The hypothalamus likely plays a critical role in this cooperative defense-driven sepsis survival since it regulates many homeostatic and endocrine pathways important during infection. To identify hypothalamic cooperative defense mechanisms promoting this survival, we conducted RNA-seq on the hypothalami of infected healthy, infected morbid, and uninfected mice then looked for genes elevated in infected healthy mice compared to both infected morbid and uninfected mice. We are currently investigating how these transcriptomic differences reveal hypothalamic cooperative defenses during sepsis.

Presenter: Gaudette, Brian

The Notch-Myc axis sets the division-differentiation clock in marginal zone B cells

Brian Gaudette, Carly Roman, Daniela GÃ³mez Atria, Ivan Maillard, David Allman

University of Pennsylvania

Lymphocyte differentiation is often tightly linked to mitosis. Clonal bursts due to antigen- or TLR-driven responses increase numbers of responding cells and may also facilitate changes in gene expression and chromatin needed for effector cell differentiation. Alternatively, to guard against rapid infection, lymphocyte pools may also contain cells poised for effector differentiation with minimal proliferation. Using cell cycle inhibitors or induced mutation of CDK1, we directly compared the impact of arresting mitosis on early plasma cell (PC) differentiation for naive follicular and marginal zone (MZ) B cells. MZ B cells reside in the marginal sinus of the spleen where they are positioned to respond rapidly to blood borne microbes. Whereas PC differentiation from follicular B cells occurred after only 4 or more divisions and was highly dependent on mitosis, MZ B cells yielded PCs much faster and despite full cell cycle blockade. Furthermore, short-term withdrawal of Notch signaling in MZ B cells in vivo caused rapid and robust down-regulation of large numbers of established Myc-regulated genes, and resulted in division/differentiation kinetics that mirrored follicular B cells. Altogether these results suggest that ongoing Notch signaling establishes a differentiation-poised state in MZ B cells needed for rapid division-independent antibody responses.

Presenter: Gerner, Romana R.

Interleukin-8 receptor-dependent B cell migration governs postnatal Peyerâ□□s patch development and confers protection to Salmonella infection

Romana R. Gerner, Suzi Klaus, Kareem Siada, Purnima Sharma, Flavian Thelen, Araceli Perez-Lopez, Marcus P. Wong, Victor Lei, Robert A. Edwards, Richard Ransohoff, Tom E. Lane, David Lo, Sean-Paul Nuccio, Elina Zuniga, and Manuela Raffatellu

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Chemokine-dependent signaling in immune cells is a major mechanism to recruit leukocytes to sites of infection or tissue damage but also guides homeostatic leukocyte trafficking and immune cell positioning. The chemokines Cxcl1 and Cxcl2 are highly up regulated in mouse cecal tissue during Salmonella-induced colitis. Its cognate receptor CXC-chemokine receptor-2 (CXCR2), also known as the Interleukin-8 receptor beta (IL-8RB), is required for neutrophil recruitment and plays critical roles in immunity. Herein, we investigated CXCR2â□□s contribution to the mucosal immune response during intestinal Salmonella infection and found a novel neutrophil-independent role for CXCR2 on B cells. In line with earlier reports, Cxcr2-/- mice exhibited a strongly impaired neutrophil recruitment to the gut resulting in increased susceptibility to Salmonella dissemination. Moreover, we observed a 100-fold higher colonization in Peyerâ□□s patches (PP) compared to wildtype littermates, which could be phenocopied by treatment with CXCR2-neutralizing antibodies, but not in mice selectively lacking Cxcr2 in granulocytes, or upon antibody-mediated neutrophil depletion. Cxcr2-/- mice did not display PP hypertrophy, a hallmark of intestinal Salmonella infection, and instead exhibited a strong decrease in PP B cell numbers. At baseline, Cxcr2-/- mice also exhibited hypoplastic PP with lower B cell numbers, but similar numbers of PP compared to wildtype mice, indicating that CXCR2 is not involved in the prenatal phase of PP formation. Mixed bone marrow (BM) chimera experiments confirmed that wildtype BM was sufficient to repopulate PP in B cell-deficient recipients (i.e., muMT-/-), whereas Cxcr2-/- BM did not result in PP formation, suggesting that CXCR2 is critical for B cell homing to PP. Furthermore, CXCR2-mediated B cell migration to PP was dependent on microbial signals, as germ-free mice developed normal PP after colonization with a low complexity microbiota, which was prevented in mice concomitantly treated with CXCR2-neutralizing antibody.Collectively, our study identifies CXCR2 as a previously unrecognized B cell receptor, and uncovers its important role in B cell homing to Peyerâ□□s patches and in host defense against a gut pathogen.

Presenter: Goldberg, Emily

Adipose tissue-resident ILC2 dysregulation underlies age-related metabolic impairments

Emily Goldberg, Irina Shchukina, Tamara Dlugos, Yoon-Hee Youm, Christina D. Camell, Maxim Artyomov, Vishwa Deep Dixit

Yale University

Aging is characterized by persistent low-grade inflammation believed to drive many chronic diseases, including insulin resistance, cardiovascular diseases, Alzheimerâ□□s disease, and immunesenescence. A primary candidate source of this increased inflammation is the increased visceral adipose tissue that accumulates during aging. The adipose-resident immune compartment is responsible for maintaining tissue homeostasis and limiting inflammation. We therefore hypothesized adipose-resident immune changes during aging were responsible for elevated inflammation leading to collapse of metabolic health. To test this, we used scRNAseq to broadly assess age-related changes exclusively in adipose-resident immune cells in young and old mice. These experiments revealed a pronounced loss of type 2 innate lymphoid cells (ILC2s), which are critical regulators of adipose tissue metabolic homeostasis. We identified dysregulation of IL-33, an important regulator of adipose ILC2s, as a driving factor leading to loss of adipose ILC2s during aging. Notably, treating old mice with IL-33 restored ILC2 numbers in adipose tissue. However, metabolic health in old mice was not improved after IL-33 treatment and actually increased susceptibility of old mice to cold challenge. Instead, using RNAseq and adoptive transfer experiments, we found that aged ILC2 are intrinsically altered during aging to become pro-inflammatory and incapable of maintaining adipose tissue homeostasis. Altogether, our work reveals a novel immune-metabolic axis that controls adipose tissue inflammation and metabolic health during aging.

Presenter: Goldman, Naomi

Exploring the mechanisms that establish the chromatin state of T cells

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

University of Pennsylvania

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-1â□□integral for normal thymic developmentâ□□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.

Presenter: Groeber, Hanna

Microbial experience influences tumor infiltrating CD8+ T cells

Hanna Groeber, Cody Morrison, Kristin Renkema
Grand Valley State University

Immune cells have been harnessed for anti-cancer therapy with varying degrees of success. One potential reason for immunotherapy failures in clinical trials may be that typical specific pathogen free (SPF) mice do not model human microbial experience. Indeed, previous studies have shown that SPF mice immunity closely resembles newborn human immunity, whereas immune systems from mice exposed to diverse microbes more closely resemble adult human immunity. We have adopted a model of microbial experience by co-housing SPF mice with mice purchased from local pet stores, therefore exposing the SPF mice to various viral, bacterial, fungal, and parasitic pathogens. Pathogen testing confirmed that the co-housed (CoH) mice are exposed to pathogens and that the SPF controls remain pathogen-free; CoH mice also gain KLRG1+ CD44+ CD8+ T cells in the blood and spleen. We injected B16 melanoma cells subcutaneously into SPF and CoH mice and monitored tumor development and T cell activation ex vivo and in vitro. CoH tumors had increased frequencies of KLRG1+ CD44+ CD8+ T cells compared to SPF tumors, and CoH tumor-infiltrating CD8+ T cells exhibited increased activation upon in vitro stimulation. Ultimately these findings will contribute to our understanding of how microbial experiences shape anti-tumor immunity and have significant implications for future immunotherapy research.

Presenter: Haecker, Hans

Pathogenic Role of Patrolling Monocytes in Lupus Nephritis

Hans Haecker, Jeeba Kuriakose, Vanessa Redecke, Cliff Guy, Jingran Zhou, Ruiqiong Wu, Sirish K. Ippagunta, Heather Tillman, Patrick D. Walker, Peter Vogel

University of Utah

Systemic lupus erythematosus (SLE) is a complex autoimmune disease that can affect almost any organ, including the kidneys. While both genetic and environmental factors contribute to disease, the pathogenic mechanisms mediating organ damage are less well-defined. Hallmarks of SLE are the appearance of immune complexes (IC) containing autoreactive antibodies and Toll-like receptor (TLR)-activating nucleic acids, whose deposition in kidney glomeruli is suspected to promote tissue injury and glomerulonephritis (GN). Here, using a mouse model based on the human SLE susceptibility locus TNIP1/ABIN1, we investigated the pathogenesis of GN. Consistent with the current model of lupus pathogenesis, we found that GN is driven by TLRs. However, inconsistent with the current model, we also found that disease proceeded independent of IC. Rather, disease in different mouse models and SLE patients was characterized by glomerular accumulation of patrolling monocytes (PMo), a cell type with emerging key function in vascular inflammation. Supporting the pathogenic relevance of these cells, monocyte-specific deletion of ABIN1 promoted kidney disease while selective elimination of PMo provided protection. Of note, PMo elimination did not protect from other disease symptoms, such as IC generation, splenomegaly and reduced survival, suggesting that GN and other inflammatory processes are governed by distinct pathogenic mechanisms. These data suggest that TLR-activated PMo represent a principal component of an intravascular process that specifically promotes glomerular inflammation and kidney injury.

Presenter: Hagan, Cassidy

Manipulating cell death pathways to promote anti-tumor immune responses

Cassidy Hagan, Annelise G. Snyder, Nicholas Hubbard, Michelle Messmer, Sigal Kofman, Brian Daniels, Andrew Oberst

University of Washington

Engaging immune responses as a strategy to treat cancer has become a major focus of modern cancer therapeutic regimens. Recently, it has been established that various mechanisms of cell death drive distinct immune responses to tumor antigens and can drastically alter the outcome of tumor progression. Inflammatory cell death modalities such as necroptosis have been shown to induce robust immune activation and tumor control while apoptosis, an immunologically silent form of cell death, can be tolerogenic. We have created systems and reagents which directly trigger immunogenic necroptosis in tumor cells in order to exploit the immunogenicity of programmed cell death. Using these, we have defined induction of necroptosis as an immunogenic event that synergizes with immune checkpoint blockade to promote durable tumor clearance. Because cell death releases cancer antigens in combination with distinct immunomodulatory signals, future treatment strategies modulating cell death pathways may benefit the immunotherapeutic goals of modern cancer therapy.

Presenter: Haldar, Malay

Tumor-derived retinoic acid promote intratumoral monocyte differentiation into immunosuppressive macrophages

Malay Haldar, Samir Devalaraja, Tsun To, and Ian Folkert

University of Pennsylvania

Tumor immunity involves capture and processing of tumor antigens by antigen presenting cells (APCs), migration of APCs to draining lymph nodes to prime T cells, and migration of primed T cells to the tumor where they exert cytotoxic anti-tumor effects (cytotoxic T cells or CTLs). APCs are important at multiple steps of this cycle; dendritic cells (DCs) process tumor antigens to prime anti-tumor T cells, DCs and immunostimulatory tumor-associated macrophages (TAMs) support CTL function, while immunosuppressive TAMs counteract CTL function. Monocytes can generate both macrophages and DCs, but within TME preferentially undergo differentiation into immunosuppressive TAMs, which is a major driver of local immunosuppression in solid tumors. However, the molecular basis of this polarized monocyte differentiation within TME is poorly understood. Using multiple murine sarcoma models, we found that TME-associated factors strongly induced retinoic acid (RA) production in tumor cells. RA promoted monocyte differentiation towards immunosuppressive TAMs and away from DCs by suppressing DC-promoting transcription factor Irf4. Reducing RA production in tumor cells or pharmacological inhibition of RA signaling engendered immunostimulatory TME, enhanced T cell dependent anti-tumor immunity, and demonstrated strong synergy with immune checkpoint blockade therapy. RA has been long considered as an anti-cancer agent, but our work demonstrates its tumorigenic capability via myeloid-mediated immune suppression and provides proof of concept for targeting this pathway for tumor immunotherapy.

Presenter: Harris, Tajie

Gasdermin-D-dependent IL-1[a] release from microglia promotes protective immunity during chronic T. gondii infection

Tajie Harris, Samantha Batista, Katherine Still, David Johanson, John Lukens

University of Virginia

Microglia, the resident immune cells of the brain parenchyma, are thought to be first-line defenders against CNS infections. We sought to identify specific roles of microglia in the control of the eukaryotic parasite Toxoplasma gondii, an opportunistic infection that can cause severe neurological disease. In order to identify the specific function of microglia in the brain during infection, we sorted microglia and infiltrating myeloid cells from infected microglia reporter mice. Using RNA-sequencing, we find strong NF-κB and inflammatory cytokine signatures overrepresented in blood-derived macrophages versus microglia. Interestingly, we also find that IL-1α is enriched in microglia and IL-1β in macrophages, which was also evident at the protein level. We find that mice lacking IL-1R1 or IL-1α, but not IL-1β, have impaired parasite control and immune cell infiltration specifically within the brain. Further, by sorting purified populations from infected brains, we show that microglia, not peripheral myeloid cells, release IL-1α ex vivo. Finally, using knockout mice as well as chemical inhibition, we show that ex vivo IL-1α release is gasdermin-D dependent. These results demonstrate that microglia and macrophages are differently equipped to propagate inflammation, and that in chronic T. gondii infection, microglia specifically can release the alarmin IL-1α, a cytokine that promotes neuroinflammation and parasite control.

Presenter: Holay, Namit

Anti-viral type I interferons induce naive-like T cells with reprogrammed metabolic signatures

Namit Holay, Barry E. Kennedy, J. Patrick Murphy, Prathyusha Konda, Michael Giaccomontonio, Joao A. Paulo, Mariam Elaghil, Gary Sisson, Youra Kim, Derek Clements, Christopher Richardson, Steven P. Gygi and Shashi Gujar

Dalhousie University

Early events during the acute phase of an immune response to virus exposure, specifically in the context of T cells, remain poorly understood. In this study, we describe the early induction of naÃ¯ve-like T cells in vivo after reovirus exposure and dissect the molecular mechanisms that drive these cells. Using a combination of mass spectrometry approaches for multiplex proteomics and metabolomics, we first identified a crucial regulatory role for type I interferon signaling in the induction of these cells after virus exposure. We further demonstrated that naÃ¯ve-like T cells had a completely rewired metabolic signature when compared to naÃ¯ve T cells. Elucidating the molecular mechanisms of early T cell phenomena after virus exposure will improve our understanding of anti-viral immunology and guide the development of viral vaccines and virus-based treatments for cancer.

Presenter: Huang, Jessica

Monocytes promote the generation of effector T cells through localized IL-12 production in draining lymph nodes

Jessica Huang, Joseph Leal, Karan Kohli, Michael Gerner

University of Washington

Cells of the innate immune system are integrally involved in the generation of adaptive immunity. Particularly, conventional dendritic cells (cDCs) are known to mediate T cell activation and differentiation in lymph nodes (LNs) during inflammation. While other innate cell subsets can contribute, their roles remain less well-defined. Here, we utilize immunization models and West Nile virus (WNV) infection, coupled with advanced microscopy approaches to study the responses of different innate cell populations in draining LNs during inflammation. We found that within hours following immunization with various type-1 inflammatory adjuvants and after WNV infection, monocytes (MOs) were rapidly recruited to draining LNs in large numbers. MOs infiltrated the draining LNs predominantly through high endothelial venules, and once in the LN, differentiated into monocyte-derived DCs (MoDCs). These MoDCs further infiltrated the deep T cell zone, and physically interacted with T cells undergoing activation by cDCs. While MoDCs did not play a major role in antigen presentation, they did constitute a significant source of IL-12 production within the T cell zone, suggesting a likely role in T cell differentiation. Indeed, blockade of monocyte trafficking into LNs with a CCR2 blocking antibody resulted in decreased IL-12 levels in the T cell zone, and in a significant reduction in fully differentiated effector CD4+ and CD8+ T cells. Interestingly, some adjuvants induced highly polarized infiltration of MOs across the lymph node, generating regions dominantly enriched in MOs and MODCs. Spatial analysis of T cell responses revealed that early-effector Tbet+TCF1- T cells were preferentially located in these MO-rich regions, while less differentiated Tbet-TCF1+ T cells were generally observed in regions relatively devoid of MOs, suggesting localized T cell differentiation. Together, these data suggest that during type-1 inflammation, monocytes infiltrate the LNs to create inflammatory microenvironments, and by cooperating with cDCs, they promote the generation of optimally differentiated effector T cells.

Presenter: Jamieson, Amanda

Innate immune adaptations of the lung epithelium alveolar macrophages, and bacterial pathogens to the Influenza A virus/bacterial coinfected environment

Amanda Jamieson, Ethan Fitzgerald, Kayla Lee, Nivea Luz

Brown University

Enter yoLower respiratory infections are among the deadliest diseases worldwide; bacterial pneumonia as a complication following influenza A virus (IAV) infection is particularly lethal. In order to survive an infection, the host must be able to clear the pathogen (resistance) and/or survive the direct damage caused by the pathogen as well as the indirect damage caused by the host immune response (tolerance). This is particularly true of the delicate and essential lung epithelium. The lung epithelium and the lung tissue resident alveolar macrophages are essential for the initial response to pulmonary infections. We have developed sequential coinfection models in air liquid interface (ALI) cultures of human bronchial epithelial cells, epithelium/macrophage coculture systems and in vivo models. These systems allow us to capture the early events that occur during IAV/bacterial coinfection. Using transcriptional profiling and imaging techniques we have investigated the alterations of both the pathogens and the host to coinfection. Cell death and immune response pathways are drastically altered during coinfection. Our transcriptional data demonstrate that there are clear changes in metabolic pathways in both the bacteria and the host during coinfection. The host metabolic changes have indications for altering the immune response, away from repair and towards inflammation. Bacteria adapt to the coinfected environment by decreasing its own metabolic pathways and increasing expression of genes important in scavenging and uptake up metabolites. These changes in the host epithelium and bacterial pathogens suggest dynamic adaptations and counter-adaptations to the increasing inflammatory pulmonary environment during coinfection. ur abstract's text here.

Presenter: Jensen, Kirk

Nfkbid-dependent B cell responses to T. gondii - new interactions revealed by a genetic screen

Kirk Jensen, Scott P Souza, Julia A Alvarez*, Samantha D Splitt*, Jessica N Wilson*, Zheng Luo#, Nicole Baumgarth#, Kirk DC Jensen**

University of California, Merced*; University of California, Davis#

Parasites are masters of immune evasion, and as such, the generation of a protective vaccine for human parasitic pathogens has been met with many setbacks. Using parasite and mouse genetic screens, our lab explores the interface between Toxoplasma gondii and the adaptive immune system. We previously reported that South American strains are particularly adept at immune evasion and cause lethal secondary infections in vaccinated or chronically infected mice. Here we asked whether immunity could be generated against such strains, and turned to mouse genetics. Unlike susceptible C57BL/6J mice, A/J mice were found to be highly resistant to secondary infection and therefore, resistance loci that segregated within 26 recombinant inbred (AxB,BxA) lines were determined. The QTL with largest effect encodes a highly polymorphic gene, Nfkbid, and found that Nfkbid null mice (bumble) were entirely susceptible to secondary, but not primary infection. Bumble mice had intact memory T cell responses, but failed to generate any parasite-specific IgM and were defective in producing most parasite-specific IgG isotypes following infection. Nfkbid encodes IκBNS, which belongs to a family of nuclear NF-κB regulators, and is required for B-1 cell development and T-independent antibody responses. Consistent with this, a unique B-1b cell expansion was observed in resistant mice that bear evidence for activation and class-switching. Moreover, we estimate nearly 70% of the antibody repertoire requires intact glycophosphatidylinositol (GPI) -lipid moieties for recognition of T. gondii surface antigens that cover the surface of most protozoan pathogens. Finally, susceptible mice fail to maintain antibody responses to GPI-lipids antigens. Taken together, we propose a model in which T cell-mediated immunity to T. gondii must be â□□layeredâ□□ with Nfkbid-driven B cell responses to GPI-associated antigens. Antibody responses to non-protein antigens may be fundamental for immunity to most parasites, and in theory, should be targeted in parasite vaccines. [*Funding: R01AI137126, R21AI145403, R15AI131027]

Presenter: Johnson, John

Repertoire Analysis of influenza-specific Tbet+ and Tbet- memory B cell pools

John Johnson, James J. Knox, Arpita Myles, Rebecca L. Rosenthal, Mariya Kostiv, Shannon R. Christensen, Jonathan Yewdell, David H. Canaday, Jinfang Zhu, Adrian B. McDermott, Yoav Dori, Max Itkin, Wenzhao Meng, Aaron Rosenfeld, Shannon Barbour, Scott E. Hensley, E. John Wherry, Norbert Pardi, Drew Weissman, Ali Naji, Michael R. Betts, Eline Luning Prak, and Michael P. Cancro

University of Pennsylvania

Antibodies that bind the conserved stalk region of influenza hemagglutinin can be broadly-neutralizing, but the B cell response that produces stalk-specific antibody is poorly understood. We have recently identified a subset of hemagglutinin-specific B cells that co-expresses the transcription factor T-bet and the myeloid lineage marker CD11c during influenza challenge that establish a long-lived memory population after infection. Using a B-lineage knockout of T-bet we have also shown that this B cell subset is required for the hemagglutinin stalk antibody response, but the relationship between T-bet+ and T-bet- hemagglutinin-specific B cells is poorly understood. To address the question of whether these memory B cell subsets are stable and separate pools we sequenced the BCRs of both subsets within individual mice after immunization. Examination of heavy-chain CDR3s shows a similar distribution of CDR3 length for both T-bet+ and T-bet- hemagglutinin-specific B cells. Moreover, both memory pools show a similar degree of somatic hypermutation which is consistent with our observations that both these subsets transit through germinal centers during the primary response. Analysis of the clonal overlap between the T-bet+ and T-bet- subsets reveals some clonal sharing, but also indicates that many clones are unique to each subset. Lineage tree analysis of the shared clones demonstrates that T-bet+ can bifurcate from T-bet- cells after somatic hypermutation and that these two subsets largely remain as separate pools after their bifurcation. We are following up on these observations using genetic fate-mapping tools to better understand the fate of T-bet+ hemagglutinin-specific B cells.

Presenter: Jung, Taylor

Identification of Immunogenic Proteins for Vaccine and Diagnostic Test Development

Taylor Jung, Catherine Terry, Roberta Pollock, Karen Molinder
Occidental College

Corynebacterium pseudotuberculosis (C. pseudotuberculosis) is a gram positive, intracellular bacterium that infects horses, causing a disease called pigeon fever. While this bacterial infection usually results in treatable external abscesses, about 8% of infected horses develop internal infections, where 30-40% of cases are fatal. There currently is no commercially available vaccine, and diagnostic methods to identify internal infections are arduous and often ineffective. Both of these factors may contribute to the high mortality rate observed with internal infections. Through multidimensional protein identification technology (MudPIT), C. pseudotuberculosis proteins recognized by antibodies from internally or externally infected horses were analyzed to identify immunogenic proteins. Of the few proteins specific to internal abscesses, carbonic anhydrase was the most immunogenic protein that was exclusively immunoprecipitated by sera from horses with internal abscesses. Enzyme Linked Immunosorbent Assays (ELISA) are in progress, to confirm that there are antibodies against carbonic anhydrase detected only in sera from horses infected with internal abscesses. If confirmed, carbonic anhydrase may be a candidate for the basis of a serum based assay as a diagnostic test for internal pigeon fever infection. Phospholipase D (PLD) was another immunogenic C. pseudotuberculosis protein identified via MudPIT. However, PLD was recognized in both sera from horses with external and internal abscesses. As PLD is a known virulence factor, however, it may be an effective protein to provide protection from C. tuberculosis infection: a formalin inactivated PLD protein vaccine was administered to mice before they were inoculated with C. pseudotuberculosis. Administration of formalin-inactivated PLD resulted in a 60% survival rate within a group of 12 mice, and thus vaccination efforts will continue with this protein. Future directions include the development of a DNA vaccine encoding proteosome-targeted mutant PLD, in an effort to trigger a Th1 response to this immunogenic protein.

Presenter: Kellar, Gerald

Increased recruitment of inflammatory monocytes contributes to age related lung pathology during respiratory syncytial virus infection.

Gerald Kellar, Katie Barrow, Kathryn L. Pothoven, Steven Reeves, Sabine Spath, and Steven F. Ziegler

University of Washington-Benaroya Research Institute

With decreasing air quality worldwide chronic pulmonary disorders are becoming an ever-increasing healthcare burden. Early life respiratory syncytial virus (RSV) infection has been linked to the onset of asthma; with no vaccine available and only one catastrophic trial attempted in the late 1960â□□s, a better understanding of the infectious progression of this pathogen is critical to better understanding its nature. The initial immune profile of RSV infected 3-week versus 8-week old C57BL/6 mice were examined to differentiate a juvenile vice adult profile, respectively; 3-week old mice displayed an enduring myeloid presence (including monocytes (MO), alveolar macrophages (AM), and neutrophils) with increased production of C-C chemokine ligand-2 (CCL2), CCL3, and CCL4 when compared to 8-week old mice at 72-hours post infection. Histological staining demonstrates that the 3-week old mice display increased accumulation of the extracellular matrix (ECM) component hyaluronan (HA) in alveolar spaces, which is traditionally increased in the asthmatic lung and can be generated and/or degraded by the MO. MO have also been linked to the exacerbation of pulmonary pathology during influenza virus infection where these cells are thought to contribute to tissue destruction while minimally contributing to viral clearance; therefore, we hypothesize that the increased recruitment of MO in the 3-week old RSV infected mice contribute to tissue remodeling that is characteristic of the progression to asthma. The specific activity of MO was examined in vitro utilizing an air-liquid interface culture involving the human U937 monocyte cell line, confirming the CCL production trend observed in the mice while further implicating matrix metalloproteinase-7 (MMP7) and MMP9 as further drivers of ECM destruction. Additionally, the enduring AM and neutrophilia presence in the 3-week old mice could further contribute to continued CCL production and general ECM dysregulation due to the nature of these cells. This data suggests that the immune profile of the juvenile lung during acute RSV infection facilitates unproductive ECM remodeling that could facilitate the conditions which support the development of asthma.

Presenter: Kongsomboonvech, Angel K.

NaÃ¯ve CD8 T cell IFN[g] responses to a vacuolar antigen are regulated by an inflammasome-independent NLRP3 pathway and Toxoplasma gondii ROP5

Angel K. Kongsomboonvech, Felipe Rodriguez, Anh L. Diep, Brandon M. Justice, Brayan E. Castallanos, Ana Camejo, Debanjan Mukhopadhyay, Gregory A. Taylor, Masahiro Yamamoto, Jeroen P.J. Saeij, Michael L. Reese, Kirk D.C. Jensen

University of California, Merced

Host resistance to Toxoplasma gondii infections rely on CD8 T cell IFNγ responses. Since manipulation of CD8 T cell IFNγ responses may influence T. gondiiâ□□s ability to achieve chronic infection, we investigated host and parasiteâ□□s requirements for eliciting this response. To this end, naÃ¯ve CD8 T cell IFNγ responses to the endogenous T. gondii vacuolar resident antigen, TGD057, were analyzed. TGD057 antigen-specific CD8 T cells (T57), isolated from naÃ¯ve transnuclear mice, responded to T. gondii-infected bone marrow-derived macrophages in an antigen-dependent manner, first by producing IL-2 and then IFNγ. T57 IFNγ responses to TGD057 antigen were independent of the parasiteâ□□s protein export machinery MYR1 and ASP5. Instead, host immunity pathways downstream of regulatory Immunity-Related GTPases (IRG), including partial dependence on Guanylate-Binding Proteins, are required. Multiple ROP5 isoforms and allele types, including â□□avirulentâ□□ ROP5A from clade A and D T. gondii strains, were able to suppress CD8 T cell IFNγ responses to parasite-infected cells. T57 response differences between clade A and B, C, D, F T. gondii strains suggest T57 IFNγ differentiation occurs independently of parasite virulence or any known IRG-ROP5 interaction. Consistent with this, removal of ROP5 is not enough to elicit maximal CD8 T cell IFNγ production to T. gondii. Instead macrophage expression of the pathogen sensors, NLRP3 and to a large extent NLRP1, were absolute requirements. In contrast, other members of the conventional inflammasome cascade are only partly required, as revealed by decreased but not abrogated T57 IFNγ responses to parasite-infected ASC, caspase-1/11 and gasdermin D deficient cells. Moreover, IFNγ production was only partially reduced in the absence of IL-12, IL-18 or IL-1R signaling. In summary, T. gondii effectors and host machinery that modulate parasitophorous vacuolar membranes, and NLR-dependent but inflammasome-independent pathways determine full commitment of CD8 T cells IFNγ responses to a vacuolar antigen.

Presenter: Kovar, Marek

IL-7/aIL-7 mAb M25 immunocomplexes expand CD8+ T cells but paradoxically abrogate the antitumor activity of CTLA-4 and PD-1 blockage

Marek Kovar, Dominik Hrabos, Tereza Hnizdilova, Jiri Uhlik

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Supraphysiological levels of IL-7 induce increase counts of pre-B cells, naive T cells and memory phenotype CD8+ T cells. Immunocomplexes of IL-7 and aIL-7 mAb M25 (IL-7/M25) were described as IL-7 superagonist in vivo. Thus, treatment of mice with IL-7/M25 remarkably increases the size of the T cell pool. We decided to use IL-7/M25 in order to expand the T cell population prior to the administration of aCTLA-4 and aPD-1 mAbs in tumor-bearing mice and in turn boost the immunotherapy based on a combination of CTLA-4 and PD-1 blockage. We found that just four doses of IL-7/M25 increased the absolute numbers of splenocytes approximately fivefold and significantly shifted the CD4+:CD8+ T cell ratio in favor of CD8+ T cells. There was also a substantive increase in relative counts of memory phenotype CD8+ T cells (approximately threefold) within CD8+ T cells but a significant decrease (approximately 30%) in relative counts of Treg cells within CD4+ T cells. All these data suggest that IL-7/M25 offer a suitable approach to potentiate tumor immunotherapy through CTLA-4 and PD-1 blockage. Unexpectedly, IL-7/M25 significantly abrogated the antitumor activity of aCTLA-4 plus aPD-1 mAbs in the following mouse tumor models: MC-38 and CT26 colon carcinoma and B16F10 melanoma. This paradoxical effect of IL-7/M25 on the antitumor activity of CTLA-4 and PD-1 blockage was not mediated via either increased levels of IL-10 or TGF-Ã□ in the sera or increased counts of IL-10-producing B or T cells in the spleen of mice injected with IL-7/M25. Thus, our work shows that caution should be exercised when combining two immunotherapy approaches together. Acknowledgement: The work was supported by the Czech Science Foundation (grant number 18-12973S) and the Institutional Research Concept RVO 61388971. The authors would like to thank Pavlina Jungrova, Helena Misurcova and Karolina Vrablova for their excellent technical assistance.

Presenter: Lacy-Hulbert, Adam

Transposon-mediated activation screening in human cells to identify mechanisms of resistance to infection

Adam Lacy-Hulbert, Anna Bruchez, Caroline Stefani, Lynda Stuart

Benaroya Research Institute

An important but understudied component of host defense is the ability of cells to resist infection, and to tolerate and repair damage caused by infection and inflammation. This process can be critical for allowing host survival while mounting effective immune responses. However, little is known about the molecular mechanisms of this resistance and resilience, and how they are initiated and regulated. We have developed a powerful forward genetic screening system, utilizing transposon mutagenesis and high throughput DNA sequencing. Unlike other approaches, this system induces both gene activation and inactivation mutations in a single screen, and is therefore complementary to more commonly used approaches such as RNAi and CRISPR. We have used this approach in a series of screens to identify new cell-autonomous mechanisms of resistance to infection and cell death. In our first screen, we investigated mechanisms of resistance to infection with Ebola virus, and identified a novel antiviral activity mediated by the NLR family transcription factor CIITA. We found that upregulation of CIITA caused resistance to infection by native Ebola virus and by EboGP-pseudotyped viruses. The antiviral activity was due to expression of a single CIITA-target gene, CD74 (Invariant chain). CD74 localizes to the endolysosome, where it binds Cathepsin proteases, preventing processing of Ebola glycoprotein and viral fusion. CIITA expression is strongly induced by IFN-gamma and we propose this mechanism provides protection of antigen presenting cells during active viral infection. In a second screen, we have identified novel genes that confer resistance to cell death induced by bacterial pore-forming toxins. We show that the endosomal protein LITAF protects cells against cell death caused by S. aureus alpha-toxin, and increases resistance to a range of other pore-forming toxins. We find that LITAF is mobilized by loss of membrane potential at sites of membrane damage, and recruits and activates the ESCRT machinery to excise and expel damaged membranes. LITAF therefore represents a critical connection between sensing of cellular damage and targeted repair. These studies have identified a number of previously unknown mechanisms of cell-autonomous protection from infection and damage. In addition, this forward genetic has identified a number of miRNA and non-coding RNA candidates which are under investigation. We believe transposon mutagenesis represents a fast and powerful approach to probe immune mechanisms that is complementary to existing reverse genetic approaches.

Presenter: Lam, Jonathan

TLR adaptors MyD88 and TRIF are critical for extrafollicular B cell responses to influenza

Jonathan Lam, Nicole Baumgarth

University of California, Davis

Antibodies produced during primary influenza infection can be protective and form rapidly. This occurs independently of germinal centers (GCs) in extrafollicular B cell (EF) responses, where antibody-secreting cells (ASCs) blast and produce antibodies in the medulla of the mediastinal lymph node. While the EF response to influenza is known to be mostly T-dependent, it is unknown why B cells are shunted away from a GC fate and what factors lead to generation of EF ASCs. We show that absence of both Toll-like receptor (TLR) adaptors MyD88 and TRIF (double knockout, DKO) cause severe, B cell-intrinsic reductions in EF responses to influenza. Observing a reduction of the B cell differentiation factor IRF4 in DKO plasmablasts in vivo, we used in vitro culture systems to assess BCR signaling and T cell help by stimulating B cells with anti-IgM(Fab)2 and CD40L and/or BAFF, respectively. DKO B cells showed reduced survival in culture, which was rescued by providing either CD40L or BAFF. Strikingly, DKO B cells barely proliferated in response to any dose of anti-IgM, with or without CD40L/BAFF. High-dose BCR stimulation further reduced the viability of DKO B cells, which correlated with reduced IRF4 induction and altered NFkB signaling. Specifically, we show by image flow cytometry reduced nuclear localization of NFkB c-Rel, a positive regulator of IRF4, following BCR stimulation of DKO compared to wild type B cells. We propose that EF responses during influenza infection require not only antigen and T cell help, but also MyD88 and/or TRIF signaling to drive activation of c-Rel. This enhances antigen-specific B cell survival, proliferation, and the strong upregulation of IRF4 required for plasmablast differentiation.

Presenter: Lau, Laura

Characterization of a novel X-linked gene implicated in Systemic Lupus Erythematosus (SLE) susceptibility

Laura Lau, Konno H, Cariaga TA, Bulloch DN, Lane J, Rapaport AS, Purtha WE, Chang AB, Rardin MJ, Gibson BW, DeVoss J, Ouyang W, Manzanillo PS

Amgen (South San Francisco)?

Systemic lupus erythematosus (SLE) is a chronic, multisystem, inflammatory autoimmune disease where up to 90% of the cases occur in women around child bearing age. Although the etiology of SLE is unknown, Type I IFN cytokines play a key role in the pathogenesis of the disease as patients with SLE have shown increased expression profile of type I IFN-regulated genes (termed the IFN signature) from blood and tissue samples. Additionally, nucleic acid-containing immune complexes recognized by the toll-like receptors (TLR), particularly TLR7 and 9, are responsible for the induction of IFNs, suggesting a key role for these receptors in driving disease. Furthermore, genome-wide association studies (GWAS) suggest there is a strong genetic component associated with SLE, but how disease-associated single-nucleotide polymorphisms (SNPs) and genetic variants affect SLE susceptibility is poorly understood. One gene that was recently identified in these GWAS studies is CXorf21, as a SNP in this gene that causes differential codon usage and increased CXorf21 expression is associated with increased susceptibility to SLE. As the structure and function of CxORF21 is unknown, we have begun investigation into its role in regulating Type I Interferon responses. Initial characterization efforts have revealed that CXorf21 is highly expressed on B cells, plasmacytoid dendritic cells (pDCs), and neutrophils, which have all been implicated as cellular drivers of SLE pathogenesis. Loss of CXorf21 in these cell types reveals an attenuated inflammatory cytokine response, namely a lack of Type I IFN production when stimulated with TLR7 and 9 ligands. Additionally, in two separate mouse models of SLE, mice lacking this gene are completely protected from disease compared to controls. Given the GWAS association of CXorf21 and our preliminary data, we hypothesize that CXorf21 is a novel protein involved in the regulation of TLR signaling and that increased expression of CXorf21 drives the IFN signature and disease progression in SLE patients.

Presenter: Leung, Gabriel

IL7R regulates fetal tissue resident macrophage development by facilitating cell survival

Gabriel Leung, Clint Valencia, Anna E. Beaudin

University of California Merced

Tissue-resident macrophages (TRMs) play critical roles in tissue homeostasis and disease. Many populations of TRMs derive from fetal progenitors and independently self-maintain across the lifespan through in situ proliferation. Previously, we have identified the interleukin-7 receptor (IL7R) as a novel regulator of TRM development. We have shown that antibody blockade of the IL7R during gestation impaired liver, lung, and epidermal TRM cellularity at birth. Here we show that in vivo fetal rIL-7 stimulation of the IL7R increased neonatal liver and lung TRM. In order to determine how IL7R signaling regulates fetal macrophage development, apoptosis was measured after late gestation IL7R blockade. Apoptosis in the macrophages and monocytes of the liver and lung was dramatically increased after IL7R blockade, suggesting that increases in neonatal cellularity after rIL-7 treatment may be due to increased survival signaling in these tissues. Our previous analysis also revealed dynamic regulation of IL7R mRNA surface protein expression as fetal monocytes differentiate into CD64+ macrophages during fetal development. We performed intracellular staining for IL7Ra protein to determine that monocytes contain intracellular IL7R protein that is not expressed on the surface until differentiation, suggesting that regulation of IL7R expression occurs at the level of surface expression. These data reveal dynamic regulation of IL7Ra expression in TRMs and TRM precursors during late gestation and provide evidence that IL-7/IL7R signaling regulates fetal TRM development by facilitating cell survival. Ongoing work addresses downstream signaling and other developmental processes regulated by IL7R signaling during fetal TRM development.

Presenter: Liu, Bo

Unc93b1 recruits syntenin-1 to control TLR7 signaling and prevent autoimmunity

Bo Liu, Olivia Majer, Lieselotte SM. Kreuk, Nevan Krogan, Gregory M. Barton

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Nucleic acid (NA)-sensing Toll-like receptors, such as TLR7 and TLR9, are tightly regulated to enable discrimination between self and foreign NAs and thus prevent autoimmunity. Despite the structural and functional similarities between these receptors, evidence from mouse models that TLR7 and TLR9 can have opposing effects in autoimmune diseases suggests that individual TLRs may have distinct regulatory mechanisms. The chaperone protein Unc93b1 mediates the trafficking of multiple TLRs from the endoplasmic reticulum to endosomes. We performed a mutagenesis screen of Unc93b1 for TLR signaling in macrophages and discovered a new function of Unc93b1 that specifically limits TLR7 but not TLR9 signaling. Mutations in Unc93b1 that lead to enhanced TLR7 signaling also disrupt binding of Unc93b1 to syntenin-1, which has been implicated in trafficking of transmembrane proteins into multivesicular bodies (MVBs). Both Unc93b1 and TLR7 can be detected in exosomes, suggesting that recruitment of syntenin-1 facilitates the sorting of TLR7 into intralumenal vesicles of MVBs, which terminates signaling. Binding of syntenin-1 requires phosphorylation of Unc93b1 and provides a mechanism for dynamic regulation of TLR7 activation and signaling. Disruption of the Unc93b1/syntenin-1 interaction in mice results in TLR7-dependent autoimmunity. Thus, Unc93b1 not only enables proper trafficking of NA-sensing TLRs but also sets the activation threshold of these potentially self-reactive receptors. Our findings suggest that Unc93b1 and its associated proteins could be potential therapeutic targets that when dysregulated can lead to chronic inflammation or autoimmunity.

Presenter: LÃ³pez, Diego A.

A critical period of innate-immune development: Fetal origins of allergic asthma susceptibility

Diego A. LÃ³pez, Anna E. Beaudin
University of California, Merced

Our lab has previously identified a developmentally-restricted hematopoietic stem cell (drHSC) that only exists during fetal development and specifically gives rise to innate-like lymphocytes that persist across the lifespan. The identification of a developmentally-limited cell of origin for innate-like immune cells that persist across the lifespan defines a â□□critical windowâ□□ for immune development, in which the phenotype of the developing immune system can be shaped via extrinsic inputs. To test how developmental perturbation during this critical window drives immune dysfunction, we examined underlying changes to innate lymphoid cells (ILCs) in the lung and susceptibility to airway dysfunction following maternal immune stimulation. ILCs are a recently identified family of fetal-derived innate-like lymphocytes that mimic the adaptive T-helper arm of our immune system. Many ILCs are tissue-resident and play essential roles in tissue development, homeostasis and protection during infection through cytokine secretion in response to tissue damage and immune activation. In the lung, type-2 innate lymphoid cells (ILC2s) are recognized as potent producers of IL5 and IL13, cytokines important for eosinophil recruitment, activation and goblet cell hyperplasia during allergic airway inflammation. Surprisingly, maternal immune stimulation via a single low-dose injection of poly (i:c) at mid-gestation robustly increased proliferative capacity and cellularity of lung ILC2s in offspring at postnatal day (P)9 and P14, respectively, concomitant with drHSC and common-helper innate lymphoid progenitor (ChILP) cell expansion during fetal development. Additionally, lung ILC2s exhibited heightened IL5 and IL13 production upon in-vitro stimulation with PMA and ionomycin in poly (i:c) perturbed offspring. Ongoing experiments will examine how heightened ILC2 functionality, as a result of developmental perturbation, underlie persistent changes at the level of the ILC-progenitor, and how this may alter susceptibility to allergic asthma in response to secondary house-dust mite immune stimulation. Together, our data suggest immune perturbation of the transient progenitors during fetal development may impact the ChILP immune trajectory, altering the establishment and function of neonatal lung ILC2s, and ultimately contributing to allergic asthma susceptibility into adulthood.

Presenter: Luban, Jeremy

HIV-1-induced cytokines deplete homeostatic innate lymphoid cells and expand TCF7-dependent memory NK cells

Jeremy Luban, Yetao Wang, Lawrence Lifshitz, Kyle Gellatly, Carol L. Vinton, Kathleen Busman-Sahay, Sean McCauley, Pranitha Vangala, Kyusik Kim, Alan Derr, Smita Jaiswal, Alper Kucukural, Patrick McDonel, Peter W. Hunt, Thomas Greenough, JeanMarie Houghton, Ma Somsouk, Jacob D. Estes, Jason M. Brenchley, Manuel Garber, Steven G. Deeks

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HIV-1 infection is associated with heightened inflammation and excess risk of cardiovascular disease, cancer, and other complications. These pathologies persist despite antiretroviral therapy (ART). In two independent cohorts, we found that innate lymphoid cells (ILCs) were depleted in the blood and gut of people with HIV-1, even with effective ART. ILC depletion was associated with neutrophil infiltration of the gut lamina propria, type 1 interferon activation, increased microbial translocation, and natural killer (NK) cell skewing towards an inflammatory state with chromatin structure and phenotype typical of WNT transcription factor TCF7-dependent memory T cells. Cytokines that are elevated during acute HIV-1 infection reproduced the ILC and NK cell abnormalities ex vivo. These results demonstrate that inflammatory cytokines associated with HIV-1 infection irreversibly disrupt ILCs. This results in loss of gut epithelial integrity, microbial translocation, and memory NK cells with heightened inflammatory potential, and explains the chronic inflammation in people with HIV-1.

Presenter: Lyons-Cohen, Miranda R.

Microanatomical Organization of Type 2 Immune Responses in the Lymph node

Miranda R. Lyons-Cohen, Michael Y. Gerner

University of Washington, Department of Immunology

Type 2 helper T (Th2) cells orchestrate a diverse range of type 2 immune responses and are critical for protection against parasitic helminths, as well as being involved in inappropriate inflammation during allergy and asthma. Substantial efforts have gone into identifying the inflammatory stimuli and in vivo mechanisms leading to the generation of Th2 cells in lymph nodes (LNs) during priming. However, these studies have largely ignored the importance of cellular localization and microenvironments in these highly organized lymphoid tissues. Here, we used highly-multiplexed quantitative imaging to study the spatial organization of early Th2 cell differentiation in skin draining LNs after Papain immunization or infection with Nippostrongylus brasiliensis. Using an IL-4 mRNA reporter (4get) OVA-specific (OT-II) adoptive transfer model, we find that Papain immunization led to extensive clustering of early Th2 cells in discrete zones located at the border of the T cell zone and B cell follicles. Similar clustering was observed for polyclonal early-Th2 cells during Nippostrongylus brasiliensis infection. Such extensive cellular aggregation was distinct from that observed during formation of Th1 responses, which instead were associated with formation of smaller T cell aggregates distributed equally throughout the T cell zone. In addition, we find that formation of Th2 zones is dependent on the site of skin immunization. Administration of Papain in the footpads elicited significantly reduced T cell clustering and Th2 differentiation in draining LNs as compared to immunization of other cutaneous sites. Collectively, these studies identify existence of dedicated microenvironments in draining LNs that support Th2 differentiation, as well as reveal that formation of these microenvironments is context-dependent, with the site of skin immunization being a critical factor in the generation of Type-2 immunity.

Presenter: Maltbaek, Joanna

Regulation of the cGAS-STING pathway through export and ENPP1-mediated degradation of cGAMP

Joanna Maltbaek, Daniel B. Stetson

University of Washington Department of Immunology

Detection of intracellular DNA by the cGAS-STING pathway activates a type I interferon-mediated innate immune response that protects from virus infection and can be harnessed to promote anti-tumor immunity. The intracellular DNA sensor cGAS binds to double-stranded DNA and synthesizes the endogenous second messenger cyclic GMP-AMP (cGAMP), which activates STING and initiates type I interferon production. While many negative regulators of this pathway have been described, the mechanisms of cGAMP regulation and degradation are poorly understood. The only identified hydrolase of cGAMP is the extracellular enzyme ENPP1, but whether cGAMP is exported to the extracellular space to enable its degradation is unclear. Congruent with this, the biological relevance of ENPP1 as a potential negative regulator of type I interferon production remains completely unexplored. Our preliminary data demonstrate that live cells potently export cGAMP in a STING-independent manner following cGAS activation. We further demonstrate that cGAMP export enables its degradation by extracellular ENPP1. Finally, we show that following acute in vivo cGAS activation, mice treated with an ENPP1 inhibitor have increased expression of interferon-stimulated genes in peripheral blood. Our ongoing studies are aimed at identifying the mechanism of cGAMP export and exploring whether ENPP1 inhibition potentiates antiviral and/or antitumor immunity. These findings will have clinical relevance for harnessing agonism of the cGAS-STING pathway for improved vaccine adjuvant design and tumor immunotherapy.

Presenter: Margolis, Shally

Functions of the STING pathway in Nematostella vectensis

Shally Margolis, Stephen C. Wilson, Russell E. Vance

UC Berkeley, HHMI

Nucleic acid sensors are critical players in innate immunity, as they allow cells to detect and respond to infection. In vertebrates, cytosolic DNA from pathogens or tissue damage is sensed by cGAS, leading to cGAMP production and STING activation. This leads to the production of type I interferons, the major cytokines that control antiviral responses. Interestingly, however, core components of the STING pathway predate the emergence of type I interferons in the vertebrate lineage. Our lab has previously demonstrated that the genome of the model cnidarian Nematostella vectensis, with which we shared our most recent common ancestor over 600 million years ago, contains functional components of the STING pathway. In this study, we aim to understand the function of this pathway in Nematostella. We have found that Nematostella express STING throughout life, and treatment of animals with cGAMP leads to the induction of putative immune genes. Some of these genes encode proteins with antibacterial activity, and their induction is dependent on the transcription factor NF- κB. In addition, some encode proteins with antiviral activity, and a subset of these seem to depend on STAT signaling. This work indicates that the ancestral functions of this pathway included both bacterial and viral control, even in the absence of interferons.

Presenter: Maurano, Megan

Uncovering the innate immune signalling pathways regulated by ADAR1 editing

Megan Maurano, Daniel B. Stetson
University of Washington

The RIG-I like receptors (RLRs) are essential to initiate the antiviral Type I Interferon response upon detection of viral RNA. However, these pathways can also be activated by endogenous RNA, resulting in autoimmunity from chronic interferon in the absence of infection. Adenosine Deaminase Acting on Double-stranded RNA (ADAR1) â□□editsâ□□ double-stranded RNAs (dsRNA) by converting adenosines to inosines, disrupting the double stranded structure and preventing aberrant MDA5 activation, interferon (IFN) production, and death. Loss of function mutations in ADAR cause aberrant recognition of self-RNA and result in the autoinflammatory disease, Aicardi-Goutieres Syndrome (AGS). 61% of patients with ADAR1-driven AGS have a P193A allele combined with a frameshift or deaminase domain mutation on the other allele. P193A (P195A in mice) is in the z-alpha domain of ADAR1, whose role in ADAR1 function is unclear. Interestingly, the prevalence of P193A far exceeds that of any other ADAR allele. To determine how P193A impairs ADAR1 function and contributes to disease, we developed a mouse model of P193A, inserting this mutation into the murine Adar locus. This new P195A mouse model more faithfully recapitulates the genotype of AGS patients than current ADAR1 loss of function models. Though homozygous AdarP195A/P195A mice are born at the normal Mendelian ratios, and survive as well as WT littermates, mice with one P195A allele combined with a null allele of either the interferon-inducible isoform of ADAR1 (AdarP195A/p150- or both isoforms of ADAR1 (AdarP195A/Adar-) have a dramatic survival defect with 100% mortality by day 120 and day 40, respectively. Prior to death AdarP195A/p150- mice are runted and have severe liver and kidney defects. AdarP195A/p150- mice are entirely rescued on an Ifih1-/- background, demonstrating that P195A specifically impacts regulation of MDA5, leaving other functions of ADAR1 intact. The survival defect and runting are entirely MDA5 dependent: AdarP195A/p150- and AdarP195A/Adar- mice are partially rescued on an Ifih1+/- background while mortality and runting are entirely prevented on an Ifih1-/- background. These mice do not have a robust IFN or ISG signature, as seen in current models, even after abrupt tamoxifen-induced deletion of the remaining Adar allele in Adarfl/P195A mice. The more subtle phenotype of these mice â□□ full knock outs of Adar and p150 are embryonic lethal - better resembles AGS, and offers opportunities to understand what pathways downstream and in parallel to MDA5 contribute to disease. Future studies will focus on how the P195A mutation affects ADAR1 localization and editing of endogenous and viral RNAs.

Presenter: Miranda, Nadia

Diet-induced weight loss restores T cell number and normalizes lipid droplets in the intestinal epithelium of obese mice

Nadia Miranda, Nadia Miranda, Natalie Limon, Christa Park, Kitty Cheung, Dr. Julie Jameson
CSU San Marcos

Diet-induced weight loss restores T cell number and normalizes lipid droplets in the intestinal epithelium of obese mice Obesity and type 2 diabetes are an increasing epidemic in the United States. Studies show that obesity causes complications such as barrier dysfunction and chronic non-healing wounds. Our laboratory has demonstrated that obesity and type 2 diabetes negatively affects the function and number of T cells in the epithelial layer of the small intestine. However, little is known about whether these T cells can be restored in number and if there is a change in lipid droplets in the intestine after a diet-induced weight loss. In this study, mice were separated into three cohorts. The control lean cohort consumed a 10% kcal Normal Chow Diet (NCD) for 14 weeks. The obese cohort consumed a 60% kcal high fat diet (HFD) for 14 weeks and the diet-induced weight loss cohort consumed a 60% kcal HFD for 7 weeks followed by a 10% kcal diet for the remaining 7 weeks. The small intestine was examined by freezing the tissue, cutting 5um sections with a cryostat machine, staining the tissue sections with antibodies specific for T cells as well as a Bodipy stain for lipid droplets, and examining the sections under an immunofluorescent microscope. Images were examined and quantified using Photoshop and ImageJ to calculate the number of T cells per villi in the small intestine and lipid droplet droplets per .7um diameter of an epithelial cell. Here we demonstrate that diet-induced weight loss restored T cell numbers and lipid droplets in the small intestine were at the same levels as a NCD cohort. This data is published and funding was provided by CSUPERB Presidents' Commission Scholars Program.

Presenter: Mohammed Salih, Mays

HnRNPA2B1 Role as an Immune Gene Regulator

Mays Mohammed Salih, Susan Carpenter
University of California/ Santa Cruz

The innate immune system is the first line of defense against pathogens; it functions through various pattern recognition receptors (PRRs) that recognize microbial products or danger signals leading to the activation of signaling pathways which initiate transcription of inflammatory genes. Activation of the innate immune response is essential to resolve infections, however, its dysregulation can result in pathological inflammation, contributing to an array of diseases, such as atherosclerosis, autoimmunity and cancer. Our understanding of the mechanisms and genes that regulate this response is not yet complete. We have recently found hnRNPA2/B1, a ubiquitous RNA processing protein, to be a negative regulator of interferon stimulated genes (ISGs) where knocking it out in macrophages resulted in an altered immune response state. We hypothesize that hnRNPA2B1 is critical for regulation of immune genes in vivo and modulates their expression in response to an infection or a stimulus. To test whether hnRNPA2B1â□□s recently discovered role as an immune gene regulator is conserved in humans, I will knock it down in macrophages differentiated from human monocyte cell line (THP) using siRNA and subject these cells to an immune challenge. Additionally, to further characterize its role in vivo specifically in macrophages â□□ integral early responders to inflammatory challenges- we recently generated a mouse where hnRNPA2B1 is exclusively knocked out in myeloid cells using a cre-lox system. Absence of hnRNPA2B1 in macrophages from mouse and human origins could result in an altered transcriptome and cytokine profile and lead to an altered ability to fight off microbes. To investigate the mechanism by which hnRNPA2B1 exerts its control on immune genes I will identify binding partners using Co-IP experiments on cells from human and mouse origins. This study will advance our understanding of how the inflammatory response is coordinated and help us better understand inflammatory and infectious diseases. Furthermore, this study will advance the field by shedding light on the functions of a poorly studied RNA processing protein thatâ□□s implicated in an array of biological processes and enhance our understanding of hnRNPs in general.

Presenter: Molina, Megan Stanley

CD8[a]+ Type 1 Conventional Dendritic Cells And Their Immediate Precursor Exhibit Phenotypic Differences And Induce Distinct Allogeneic CD8+ T-Cell Responses

Megan Stanley Molina, Jessica Stokes, Emely Hoffman, Richard J. Simpson, Emmanuel Katsanis

University of Arizona

The type 1 conventional murine dendritic cell (DC) subset, CD8α+ cDC1, has been identified as a strong inducer of anti-cancer responses through cross-presentation of tumor antigens to CD8+ T-cells. CD8α+ cDC1s have also been identified as suppressors of graft-versus-host disease (GvHD) in the context of allogeneic bone marrow transplantation (BMT). The immediate precursor to this DC subset, pre-cDC1, has been identified as CD24highCD8α-. The few studies characterizing this precursor population have found that pre-cDC1s more potently induce memory CD8+ T-cells in response to viral infection compared to CD8α+ cDC1, indicating fundamental functional differences. However, this precursor population has never been studied in the context of alloreactivity as compared to CD8α+ cDC1s. We therefore used flow cytometry to evaluate the expression of activation markers and inhibitory signaling molecules on pre-cDC1 as compared to CD8α+ cDC1 in naÃ¯ve mice and in mice treated with sub-lethal total body irradiation (TBI). We further compared the ability of pre-cDC1 and CD8α+ cDC1 to stimulate allogeneic, naive total T-cells and allogeneic, naÃ¯ve CD8+ T-cells. We report that pre-cDC1s exhibit significant differences in their expression of the activation and inhibitory molecules PD-L1, CD70, ICOSL, and PIR-B compared to CD8α+ cDC1s. Further, we report that stimulation of allogeneic T-cells with pre-cDC1 compared to CD8α+ cDC1s results in significantly more total T-cell proliferation measured by tritiated thymidine uptake. When we further analyzed the ability of pre-cDC1s to stimulate allogeneic CD8+ T-cells compared to CD8α+ cDC1s, we find that stimulation with pre-cDC1s result in significantly higher expression of CD44, a marker for effector memory T-cells, and significantly higher expression of CD122 (IL-2RB). Additionally, stimulation with pre-cDC1s results in a trend of greater expression of TIM-3, a marker of T-cell exhaustion. These results indicate significant phenotypic and functional differences between these two subsets of murine DCs, though further studies are required to determine the role pre-cDC1s may play in the context of GvHD and anti-cancer responses.

Presenter: Morawski, Peter

A circulating population of CD4+CD103+ cutaneous resident memory T cells is reduced during human fibrotic disease

Peter Morawski, Mitch Fahning, Suraj Varkhande, Emma Beale, Maria Klicznik, Iris Gratz, Dan Campbell

Benaroya Research Institute at Virginia Mason

Tissue-resident memory T cells (TRM) persist mainly in non-lymphoid tissues where they promote immune recall responses to recurrent insults. We previously defined a population of CD4+CD103+ TRM cells from human skin that become CD69neg and re-enter the circulation, but maintain expression of the skin-associated marker cutaneous lymphocyte antigen (CLA) and the capacity to return to the skin. Comparing blood- and skin-derived CLA+ memory populations, we found that the recirculating fraction of CD103+ TRM are clonally, transcriptionally, and phenotypically most similar to skin-resident CD103+ T cells. We now report that the circulating CD4+CLA+CD103+ T cells are functionally distinct from classically-defined CD4+ T helper lineages, highlighted by the co-production of IL-22 and IL-13. As these cytokines can promote tissue repair macrophage- and epithelial cell-mediated fibrosis and wound healing, we assessed the circulating skin TRM pool during systemic scleroderma (SSc), an autoimmune disorder highlighted by fibrosis of the skin and the underlying organs. Compared to healthy donors, SSc patients had a reduced frequency of circulating CD4+CLA+CD103+ T cells with diminished per-cell IL-13 production, both of which correlated with late-onset disease. Our ongoing work aims to understand how changes in the circulating TRM fraction might be a litmus for the onset or progression of SSc and similar skin diseases. Therefore, circulating CD4+CLA+CD103+ T cells represent a broadly available pool from which the isolation and study of skin TRM cell responses is possible both in health and disease.

Presenter: Mullins, Genevieve

T cell signaling and Treg dysfunction define disease kinetics in IL-2Ra-KO autoimmune mice

Genevieve Mullins, Kristen M. Valentine, Mufadhal Al-Kuhlani, Dan Davini, Kirk D.C. Jensen, Katrina K. Hoyer

UC Merced

IL-2Rα is required to generate the high affinity receptor for IL-2, a cytokine important in immune proliferation, activation, and regulation. Mice deficient in IL-2Rα (IL-2Rα-KO) develop systemic autoimmune disease and die from severe anemia between 18-80 days of age. These mice develop kinetically distinct autoimmune progression, with approximately a quarter dying by 21 days of age and half dying after 30 days. This research aims to define immune parameters and signaling that distinguish cohorts of IL-2Rα-KO mice that develop early- versus late-stage autoimmune disease. To investigate these differences, we evaluated complete blood counts (CBC), antibody binding of RBCs, T cell numbers and activation, hematopoietic progenitor changes, and signaling kinetics, during autoimmune hemolytic anemia (AIHA) and bone marrow failure. Using a simple CBC we were able to predict disease kinetics to explore mechanisms underlying early and late disease. We identified several alterations, that combined, contribute to disease kinetics. Early onset disease correlates with anti-RBC antibodies, lower hematocrit, reduced IL-7 signaling, and increased CD8 T cell expansion. CD8 regulatory T cells (Tregs) lost IL-10 expression and had enhanced apoptosis in early disease. Further, CD8 Tregs maintained a higher suppressive capacity and presence in delayed disease. IL-2Rα-KO disease pathology rates are driven by T cell signaling that skew effector T cell activation and expansion, and Treg dysfunction. Altered cytokine and TCR signaling, from IL-2RÃ upregulation and increased IL-2 production, along with decreased suppression from CD8 Tregs, may in turn promote increased CD8 T cell expansion, resulting in the elevated CD8 T cells and effector/memory fate choices that skew disease kinetics.

Presenter: Murthy, Aditya

The autophagy gene Atg16L1 constrains cytosolic anti-microbial immunity by suppressing oxidative stress

Aditya Murthy, Timurs Maculins, Erik Verschueren, Trent Hinkle, Patrick Chang, Cecile Chalouni, Junghyun Lim, Anand Kumar Katakam, Mike Reichelt, John Rohde, Ivan Dikic, Donald Kirkpatrick

Genentech, Inc.

Defective autophagy is strongly linked to chronic inflammatory diseases. Human genetics revealed that loss-of-function of the core autophagy gene ATG16L1 increases risk for Crohnâ□ □s disease in part by enhancing innate immunity in mucosal tissues. This observation prompted a re-evaluation of the role of ATG16L1 in the anti-microbial immune response against cytosolic pathogens. Shigella flexneri (S.flexneri) exemplifies an enteric bacterium capable of escaping membrane-bound compartments including autophagosomes to reside and expand within the macrophage cytosol following infection. In this study, we found that loss of ATG16L1 unexpectedly enhanced the killing of virulent S.flexneri (M90T) by macrophages. Quantitative multiplexed proteomic analysis revealed that ATG16L1 deficiency resulted in upregulation of proteins involved in antioxidant response to compensate for elevated oxidative stress and drove S.flexneri killing in primary murine macrophages lacking ATG16L1. Myeloid cell-specific deletion of Atg16l1 was also sufficient to accelerate bacterial clearance in vivo. Finally, we demonstrated that pharmacological inhibition of cystine import to increase oxidative stress conferred enhanced microbicidal properties to wild-type macrophages. These findings reveal that control of oxidative stress by ATG16L1 and autophagy regulates anti-microbial immunity against cytosolic pathogens.

Presenter: Nadjsombati, Marija

Regulation of succinate-driven type 2 immune responses in the small intestine

Marija Nadjsombati, John McGinty, Natalie Niepoth, AndrÃ©s Bendesky, Jakob von Moltke
University of Washington

In the small intestine, type 2 immune responses can be initiated through a tuft-ILC2 circuit in which tuft cell-derived IL-25 activates group 2 innate lymphoid cells (ILC2s) in the lamina propria. Recently, our group and others reported that tuft cells in the small intestine detect Tritrichomonas protists via their secreted metabolite succinate. Succinate binding to its receptor, SUCNR1, on tuft cells initiates a signaling cascade sufficient to induce a robust type 2 immune response, particularly in the distal small intestine (i.e. ileum). However, while studying succinate sensing by tuft cells, we observed that activation of the tuft-ILC2 circuit, as measured by tuft cell hyperplasia, varies among different strains of mice. Balb/c mice do not develop tuft cell hyperplasia when given succinate yet are capable of responding to succinate if first â□□primedâ□□ with recombinant IL-25. This suggests all components of the succinate-sensing tuft-ILC2 circuit are functional, yet differentially regulated between B6 and Balb/c mice. We demonstrate that this differential regulation is genetically encoded. Balb/c x B6 F1 progeny all develop tuft cell hyperplasia following succinate treatment and Balb/c x B6 F2 progeny have varied responses with approximately a 3 to 1 ratio of responder to non-responder mice. Furthermore, quantitative trait loci (QTL) mapping of succinate response phenotypes of Balb/c x B6 F2 mice identified a single robust QTL associated with variations in succinate responsiveness. This QTL does not contain any genes currently implicated in the tuft-ILC2 circuit. Together, these results indicate that a single recessive locus in Balb/c mice regulates succinate-mediated activation of the tuft-ILC2 circuit. We hypothesize that there is a novel regulator of the succinate-tuft-ILC2 circuit and future work will aim to define the mechanism by which this regulation occurs.

Presenter: Nguyen, Trang

Reversing T cell anergy by removing E3 ubiquitin ligases Cbl-b.

Trang Nguyen, Zhi-En Wang, Lin Shen, Arthur Weiss.
University of California, San Francisco

T cell anergy is an intrinsically inactive state resulting from absent co-stimulation and/or high co-inhibitory tone. T cell anergy is an important peripheral tolerance mechanism to prevent self-reactive T cells from attacking host tissues. Unresponsiveness in anergic T cells has been attributed to the induction of multiple negative regulators, including E3 ubiquitin ligases, that target T cell receptor (TCR) signaling pathways. However, it is not generally understood how T cell anergy states are established and maintained. We generated a model of T cell anergy by introducing the Zap70 hypermorphic mutant, W131A, into the OT2 transgenic background (W131AOT2) which resulted in high numbers of anergic and Treg CD4 T cells. Peripheral W131AOT2 anergic CD4 T cells had impaired TCR signaling and failed to produce IL-2 or up-regulate CD69, CD25, and Nur77 after antigen or TCR stimulation. Interestingly, single positive CD4 thymocytes from these mice exhibited normal TCR signaling and normal upregulation of CD25, CD69, and Nur77, as well as IL2 production in response to antigen or anti-CD3 stimulation. Thus, the anergic phenotype is acquired in the periphery. The E3 ubiquitin ligase Cbl-b (Cbl Proto-Oncogene B) is highly expressed in anergic T cells and contributes to establishing the unresponsive state. Cbl-b-/- T cells are largely resistant to T cell anergy induction and Cbl-b-deficient (Cbl-b-/-) mice have enhanced susceptibility to develop spontaneous autoimmunity. Thus, Cbl-b plays a central role in the development of autoimmunity and setting a threshold for T cell activation. Peripheral W131AOT2 T cells had large increases in Cbl-b mRNA and protein expression compared to control OT2 T cells. To assess functions of Cbl-b in maintaining T cell anergy, W131AOT2 were crossed to Cbl-b deficient (Cbl-b-/-) mice to generate W131AOT2 Cbl-b-/- mice. Interestingly, W131AOT2 Cbl-b-/- exhibited similar frequencies of phenotypically anergic, Treg CD4 T cells compared to W131AOT2 mice. However, loss of Cbl-b in W131AOT2 mice reversed peripheral T cell unresponsiveness to antigen or anti-CD3 stimulation, including up-regulation of phosphorylated Erk (pErk), phosphorylated Akt (pAkt), T cell activation markers CD69, CD25, and the proportion of IL-2 secreting CD4 T cells and their proliferation. Together, these results reveal that T cell anergy is induced in the periphery and Cbl-b plays an essential role in the regulation of peripheral tolerance and anergy of T cells.

Presenter: Nice, Timothy

Selective interferon responses of intestinal epithelial cells minimize TNFa cytotoxicity.

Timothy Nice, Jacob Van Winkle, David Constant, Lena Li

Oregon Health & Science University

Interferon (IFN) family cytokines stimulate genes (ISGs) that are integral to antiviral host defense. Type I IFNs act systemically whereas type III IFNs act preferentially at epithelial barriers. Among barrier cells, intestinal epithelial cells (IECs) are particularly dependent on type III IFN for control and clearance of virus infection, but the physiological benefit of this selective IFN response is not well understood. Here, we find that in vivo neonatal IECs are minimally responsive to type I IFN whereas in vitro IEC organoids respond robustly. In contrast, neonatal and organoid IECs are equivalent in their response to type III IFN, suggesting specific in vivo suppression of type I IFN responses. Type I IFN treatment of responsive IEC organoids stimulates expression of more genes than type III IFN, including pro-apoptotic genes that amplify tumor necrosis factor (TNF)-triggered cytotoxicity. The expanded set of type I IFN-stimulated genes have sub-optimal interferon-stimulated response element (ISRE) promoter motifs whereas ISGs stimulated in common by types I and III IFN have optimal ISRE motifs. Thus, preferential responsiveness of IECs to type III IFN enables â□□optimalâ□□ antiviral ISG expression but minimizes expression of â□□sub-optimalâ□□ ISGs that have potential to disrupt intestinal homeostasis during infection or inflammation.

Presenter: Olsen, Tayla M

Caspase deficiency leads to STING-dependent, type I IFN-independent immune cell dysregulation

Tayla M Olsen, Arne Knudsen, Allie Kehret, Anthony Rongvaux

Fred Hutchinson Cancer Research Center

Caspases are evolutionarily conserved proteases that play a central role in cell death and therefore, cellular and tissue homeostasis since the mechanism by which a cell dies instructs both the innate and adaptive immune system. Caspase-9 (Casp9) and Casp3/7 are well-known mediators of apoptotic cell death, triggered downstream of mitochondrial stress. However, their role in immune regulation is incompletely understood. Inhibition or genetic knock-out of Casp9 or of Casp3/7 leads to spontaneous STING-dependent type I interferon (IFN) production, indicating that these caspases also have a role in regulating innate immunity. Here, we show that mice deficient for Casp3/7 or Casp9 in their immune compartment, have reduced number and impaired activation of natural killer (NK) cells. This is a cell-extrinsic phenotype brought about independently of type I IFN signaling but is partially STING-dependent, suggesting a type I IFN-independent role for STING. Here, we investigate the cellular source of this phenotype in vivo and investigate the non-apoptotic roles of Casp3/7/9 in maintaining cellular homeostasis and how loss of these caspases leads to the observed phenotype. Dysregulated cell death is associated with many pathologies and our work may provide new insights into how caspases participate in disease pathogenesis.

Presenter: Oyebola, Oyesola

The prostaglandin D2 receptor CRTH2 promotes IL-33-induced ILC2 accumulation in the lung

Oyesola Oyebola, Carolina Duque, Linda C. Huang, Elisabeth M. Larson, Simon P. Fr?h, Lauren M. Webb, Seth A. Peng, and Elia D. Tait Wojno

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ILC2s are rare innate immune cells that accumulate in tissues during allergy and helminth infection, performing critical effector functions that drive type 2 inflammation. ILC2s express ST2, the receptor for the cytokine interleukin-33 (IL-33), and chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2), a receptor for the bioactive lipid prostaglandin D2 (PGD2). The IL-33-ST2 and the PGD2-CRTH2 pathways have both been implicated in promoting ILC2 accumulation during type 2 inflammation. However, whether these two pathways coordinate to regulate ILC2 population size in the tissue in vivo remains undefined. Here, we show that ILC2 accumulation and associated type 2 inflammation in the murine lung in response to systemic IL-33 treatment were partially dependent on CRTH2. This effect was not a result of reduced ILC2 proliferation, increased apoptosis or cell death, or differences in expression of the ST2 receptor in the absence of CRTH2. Rather, data from adoptive transfer studies suggested that defective accumulation of CRTH2-deficient ILC2s in response to IL-33 was due to altered ILC2 migration patterns. While donor wild type ILC2s preferentially accumulated in the lungs compared to CRTH2-deficient ILC2s following transfer into IL-33-treated recipients, wild type and CRTH2-deficient ILC2s accumulated equally in the recipient mediastinal lymph node. These data suggest that CRTH2-dependent effects lie downstream of IL-33, directly affecting the migration of ILC2s into inflamed lung tissues. A better understanding of the complex interactions between the IL-33 and PGD2-CRTH2 pathways that regulate ILC2 population size will be useful in understanding how these pathways could be targeted to treat diseases associated with type 2 inflammation.

Presenter: Palaferri Schieber, Alexandria

Vitamins in Host Defense Against an Enteric Pathogen

Alexandria Palaferri Schieber, Janelle Ayres

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Upon infection, the host mounts multifactorial responses to defend against disease. Host defense can be categorized as resistance, disease tolerance, or anti-virulence defenses. While resistance mechanisms encoded by the immune system kill the pathogen, disease tolerance and anti-virulence defenses promote host health while having a neutral to positive effect on pathogen fitness and are executed by the cooperative defense system. Anti- virulence mechanisms are physiological responses of the host that prevent or neutralize pathogenic signals, whether from the pathogen or from the host response to the pathogen. Disease tolerance involves mechanisms that induce health by decreasing tissue susceptibility to these pathogenic signals. One known regulator of immunity is the calcitriol receptor, commonly known as the Vitamin D receptor (VDR). Utilizing multiple murine models to investigate the role of VDR during infection with an attaching and effacing (A/E) pathogen, our data implicate the VDR in disease tolerance or anti-virulence defenses in response to infection. Intact Calcitriol receptor promotes host health during infection but does not alter pathogen burden. We are currently investigating how this pathway mediates cooperative defenses against infection focusing on VDRs role in glucose metabolism and organ homeostasis.

Presenter: Perez, Oriana

Macrophage-pDC dynamics and interferon production during viral infection

Oriana Perez, Stephen T. Yeung, Kamal Khanna, Mohamed Oukka, Daniel Stetson, Boris Reizis
NYU Medical School

Plasmacytoid dendritic cells (pDCs) are a specialized subset of dendritic cells that produce robust quantities of type I interferons (IFNs) early after viral infection. The early production of IFNs is critical for initiating a series of antiviral host responses driven in part through the activation of innate immune cells and the formation of adaptive immunity. Previous in vitro studies have shown that IFN production by pDCs can be triggered by interaction with live virus-infected cells, however there is currently no evidence demonstrating pDC activation in vivo. Thus, the exact cellular dynamics regulating pDC activation in vivo are not fully understood. To address this current gap in our understanding we sought to visualize pDC activation in vivo following viral infection. For this purpose we utilized novel reporters to visualize interferon beta (IFNB) responses and pDC dynamics following local vesicular stomatitis virus (VSV) skin infection. Our recent studies showed that CD169+ macrophages in the skin draining lymph nodes (LNs) regulated pDC activation and IFNB responses. For these studies we locally depleted CD169+ macrophages (CD169-DTR mice) and showed that in the absence of CD169+ macrophages pDC activation was significantly impaired following VSV infection. Histological analysis suggests pDCs in close proximity to VSV-infected CD169+ macrophages were the primary producers of IFNB and in the absence of CD169+ macrophages the spatial organization of IFN?-producing pDCs was altered. These finding reveal a dynamic functional dichotomy between the role of macrophages and pDCs in host protection against viral infection.

Presenter: Pollock, Tzvi

Determining the Mechanism of TNF-Mediated Defense Against Legionella pneumophila Infection

Tzvi Pollock, Sunny Shin

University of Pennsylvania

Intracellular bacterial pathogens are responsible for significant disease burden every year. Successful control of these organisms by the host depends on the inflammatory cytokine Tumor Necrosis Factor (TNF). While TNF is known to protect against many intracellular bacterial infections, and the molecular mechanisms of TNF signaling are understood in sterile contexts, the precise modes through which TNF can defend the host against bacterial infection remain unclear. The following research aims to elucidate the mechanism of TNF in the context of intracellular infection. In this project, the gram-negative bacterium Legionella pneumophila, the causative agent of Legionnaireâ□□s Disease, acts as a model intracellular pathogen. Infection of bone marrow derived macrophages and live mice are both used to explore the effect of TNF signaling on control of infection. Thus far we have shown that TNF is required for restriction of bacterial replication both in vitro and in vivo. In addition, the cysteine protease caspase 8 appears to be necessary for control of Legionella infection in vivo, however its pro-apoptotic auto-processing does not. Preliminary data suggest that caspase 8 still mediates a form of TNF-dependent cell death in response to infection. Thus, a function of caspase 8 other than classical apoptosis may therefore be the mechanism through which TNF restricts bacterial replication.

Presenter: Pothoven, Kathryn

Therapeutic targeting of airways epithelium for the prevention of rhinovirus induced asthma exacerbation

Kathryn Pothoven, Rane S. Creasy BSc, Kaitlyn A. Barrow BSc, Matthew C. Altman MD, Jason S. Debley MD, Steven F. Ziegler PhD

Bnaroya Research institute

Human rhinovirus (HRV) infection is a common viral trigger of asthma exacerbation. Airway epithelial cells express ICAM1, the entry receptor for the A serotype of HRV and they are the first cell-type to encounter HRV upon infection. Epithelial cells play an important role in general tissue homeostasis, repair of tissue injury, and responses to inflammatory stimuli. Additionally, epithelial cells from asthmatic patients have been shown to be structurally dysfunctional compared to healthy epithelia. We hypothesize that the airway epithelial response to HRV primes a subsequent asthma exacerbation and that the clinical timepoint of onset of rhinovirus symptoms may be a potential therapeutic intervention point aimed at preventing or reducing the symptoms of rhinovirus induced exacerbations. Airway epithelial cells were obtained from control (H) and asthmatic pediatric patients. The asthmatic patients can be further categorized based on whether the patient has a previous history of exacerbation. Patients with a previous history of exacerbation (E) have more severe disease that patients that do not have a history of exacerbation (A). The nasal epithelial brushings from all patients were grown at air-liquid interface (ALI) and they were either left uninfected or infected with HRV16 and at 24 hours post infection RNA was harvested for RNAseq analysis. A Venn diagram comparing the H, A and E groups showed 36 increased and 43 decreased genes in the H group, 34 increased and 39 decreased genes in the A group, and 475 increased and 389 decreased genes in the E group. The E group is distinct from the other two groups, supporting our hypothesis that the epithelial cells from patients with a history of previous exacerbation may prime a subsequent exacerbation. Clustering analysis of the 475 upregulated genes in the E group identified a unique cluster of cytokines and cytokine receptors, OSMR, IL1A, IL4R, INFGR2, IL23A, IFNL1, IFNL2, and IFNL3 that are attractive therapeutic candidates. Mechanistic studies will be conducted to determine the efficacy of inhibition of these therapeutic candidates in the context of airways disease.

Presenter: Radu, Caius

Inhibiting adenosine signaling and KRAS enhances the effect of a-PD-1 therapy in a KRASG12C/TP53R172H/+ pancreatic cancer model

Caius Radu, Author Names s Thuc M Le, Evan Abt, Woosuk Kim, Akshata Udyavar, Arthur E Cho, Joe Capri, Jing Cui, Razmik Ghukasyan, Luyi Li, Brandon Rosen, Timothy R Donahue, Daniel DiRenzo, Matthew J Walters

University of California Los Angeles

Introduction: High levels of adenosine (ADO) in the tumor microenvironment have been shown to suppress immune responses and curtail T cell activation in the presence of anti-PD-1/PD-L1 blocking antibodies. CD73 catalyzes the extracellular generation of ADO from adenosine monophosphate (AMP). KRAS mutations, of which 60% were derived from pancreatic ductal adenocarcinoma (PDAC) samples, were associated with significantly upregulated CD73 expression, which resulted in a worsening prognosis. These immunosuppressive effects can be counteracted by CD73 inhibitors or by a dual ADO receptor (A2aR/A2bR) antagonist.? Methods: Linear models were used to evaluate the ability of 299 pan-cancer consensus oncogenic drivers to predict CD73 expression independent of tumor type in the TCGA dataset. Changes in gene expression induced by KRAS inhibition were determined by RNAseq. Metabolic and proteomic alterations induced by KRAS inhibition were determined by LC-MS. C57BL/6J mice bearing established KP4662-G12C (KRASG12C/TP53R172H/+) tumors (at least 150 mm3 in volume) were treated as follows: A1421 (CD73i; 30 mg/kg/day, s.c.), anti-PD1 (Clone RMP 1-14; 10 mg/kg; twice per week, i.p), and MRTX-1257 (KRASi, 100 mg/kg/day, p.o.). Treatment efficacy was monitored in a blinded manner using micro-computed tomography (mCT). A1412 and MRTX-1257 were provided by Arcus Biosciences.? Results: Direct KRAS inhibition reduced but did not abolish CD73 and A2aR/A2bR expression in multiple PDAC models. Metabolic analyses indicated that KRAS inhibition increased ADO and AMP levels. KRAS inhibition in PDAC models induced gene expression changes consistent with increased tumor immunogenicity. In a murine model of pancreatic cancer-bearing the KRASG12C mutation, co-administration of a CD73 inhibitor with anti-PD-1 in established tumors resulted in significant tumor growth retardation, comparable to KRASG12C inhibition alone. Durable tumor regression was observed when mice were treated with the triple combination therapy. These data support the rationale for the clinical development of modulators of immunosuppressive adenosine signaling in pancreatic cancer.? Conclusion: Here, we show that direct inhibition of mutant KRAS in pancreatic cancer models yields complex immunomodulatory effects. While antigen presentation pathways are transcriptionally upregulated and the expression of immunosuppressive chemokine is reduced, KRAS inhibition also reprograms nucleotide metabolism leading to elevated levels of ADO. These findings suggest that co-targeting mutant KRAS and adenosine signaling may enhance immunotherapy against pancreatic cancer and potentially other RAS driven malignancies.

Presenter: Rajan, Malini

Coupling intestinal iron uptake and innate immunity in C. elegans

Malini Rajan, Malini Rajan, Cole P. Anderson, Paul M. Rindler, S. Joshua Romney, Maria C. Ferreira dos Santos, Jason Gertz, Elizabeth A. Leibold

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Iron is essential for survival of most organisms, but is toxic in excess. All organisms have thus developed mechanisms to sense, acquire and sequester iron. In C. elegans, iron uptake and sequestration are regulated by HIF-1. We previously showed that hif-1 loss of function mutants are developmentally delayed when grown under iron limitation. Here we identify nhr-14, encoding a nuclear receptor homologous to vertebrate HNF4, in a genetic screen conducted for mutations that rescue the developmental delay of hif-1 mutants under iron limitation. NHR-14 is highly expressed in intestinal cell nuclei and in cells in the head, and its subcellular localization and expression are not regulated by iron. Loss of nhr-14 leads to the upregulation of the intestinal metal transporter SMF-3 that increases iron uptake in hif-1 mutants, rescuing the low iron-dependent developmental delay. Loss of nhr-14 also promotes the nuclear localization of the zinc-finger transcription factor PQM-1, which activates smf-3 through the interaction with GATA-like DAF-16-associated elements (DAEs) in the smf-3 promoter. In addition to smf-3, RNA-seq analysis revealed upregulation of innate immune response genes as well as DAF-16/FoxO-suppressed Class 2 genes, which are known to be regulated by PQM-1. Consistent with the upregulation of innate immune response genes, nhr-14 mutants showed enhanced resistance to the human pathogen Pseudomonas aeruginosa that depends in part on the upregulation of smf-3 and iron uptake as well as the upregulation of innate immune response genes. P. aeruginosa reduces expression of nhr-14 in wild-type N2 worms, which is associated with PQM-1 nuclear localization. We propose that increased iron uptake by SMF-3 is a strategy to limit the acquisition of intestinal iron by pathogens, and may serve as a critical component of the host innate immune response. Our data provide insight into how C. elegans utilizes nuclear receptors to regulate innate immunity and iron availability, and show iron sequestration as an important component of the innate immune response.

Presenter: Ripperger, Tyler

Transcriptional Regulation of Durable Antibody Mediated Immunity

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Durable antibody-mediated immunity is maintained by memory B cells and long-lived plasma cells. The length of protection varies greatly depending on the specific vaccine or infection. Defining the transcriptional profiles required for sustained antibody-mediated immunity may help explain these differences and provide guidance on how to improve vaccine responses. Previously, our lab demonstrated two members from the BTB/POZ family of transcription factors exert opposite effects on the duration of antibody responses and plasma cell longevity. ZBTB20 is required for long-term primary antibody responses and plasma cell longevity, while the related ZBTB32 suppresses the lifespan of plasma cells in recall responses. We hypothesize ZBTB20 and ZBTB32 regulate the magnitude of antibody responses and plasma cell lifespan through temporal cell type specific mechanisms. We will test our hypothesis by: 1) Establishing the B cell stages in which ZBTB20 is required; 2) Determining direct targets and genes regulated by ZBTB20 and ZBTB32. These experiments will define essential genetic programs that promote and antagonize plasma cell lifespan.

Presenter: Robinson, Elektra

Inflammation drives alternative first exon usage to regulate immune genes including Aim2

Elektra Robinson, Pratibha Jagannatha, Sergio Covarrubias, Matthew Cattle, Rojin Safavi, Ran Song, Kasthuribai Viswanathan, Barbara Shapleigh, Robin Abu-Shumays, Miten Jain, Suzanne Cloonan, Edward Wakeland, Mark Akeson, Angela N. Brooks and Susan Carpenter

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Macrophages are critical effector cells of the innate immune system essential for controlling infection and maintaining tissue homeostasis. At the cellular level, pathogen-response involves recognition by classes of receptors expressed on the cell surface and intracellularly, once activated they initiate complex signaling cascades that result in the induction of an inflammatory program. Perturbations to these signaling pathways can have devastating consequences, leading to autoimmune diseases and cancer. The contribution of alternative splicing to the regulation of innate immune responses remains poorly studied. Through the analysis of differential splicing using both short read and long-read RNA sequencing of human and mouse macrophages, we have identified that alternative first exon (AFE) changes are a prominent widespread event during inflammatory activation. Of these AFE events, we have identified 50 unannotated transcriptional start sites (TSS) in mouse bone marrow-derived macrophages (BMDMs) using Oxford Nanopore technology, one of those being Aim2. The protein Aim2 (Absent in Melanoma) is the cytosolic receptor for dsDNA within the cytosol. Once Aim2 is activated by dsDNA it results in the formation of the inflammasome complex leading to the production of the pro-inflammatory cytokines IL1b and IL18. From a combination of high throughput approaches including ChIP-seq, ATAC-seq, and RNA-seq we characterized the unannotated promoter and TSS of Aim2 as inflammatory specific. Next, we examined the 5â□□UTR of the unannotated Aim2 isoform and identified an iron response element (IRE). Our functional data of polysome profiling, GFP reporter system and western blot analysis indicate that this unannotated inflammatory activated isoform is translated at a lower level compared to the canonical isoform. This result is also reversible through the manipulation of iron or the removal of the IRE. This could be a mechanism required in order to limit activation of the Aim2 pathway so that following inflammation Aim2 protein activation is tightly controlled and rapidly switched off. This novel isoform of Aim2 is conserved between human and mouse. We are now determining the exact mechanisms of action and regulation of this isoform and its importance in autoimmune diseases such as Systemic Lupus Erythematosus.

Presenter: Rodriguez, Felipe

Three genetic loci of Toxoplasma gondii determine whether naÃ¯ve CD8 T cells make IFN[g] to genetically divergent parasite strains

Felipe Rodriguez, Angel K Kongsomboonvech, Kirk DC Jensen

University of California, Merced

Host survival to Toxoplasma gondii infection is dependent upon CD8 T cell IFNγ responses. Since manipulation of CD8 T cells may influence T. gondiiâ□□s ability to achieve chronic infection, we asked whether the parasite modulates activation of this cell type. To address this, we analyzed naÃ¯ve CD8 T cell responses to the endogenous, vacuolar resident antigen, â□□TGD057â□□. NaÃ¯ve TGD057 antigen-specific CD8 T cells (T57) were isolated from transnuclear mice and assayed for their ability to secrete IFNγ to T. gondii-infected bone marrow derived macrophages. A unique phenotypic pattern emerged in which CD8 T cells responded vigorously to all T. gondii strains, except those from clade A, suggesting the presence of novel polymorphic virulence factors in which we named Regulator Of CD8 T cell Responses (ROCTR). In order to identify ROCTR a genetic mapping experiment was performed by analyzing T57 IFNγ responses to 35 progeny from a genetic cross between a clade A (low inducer) and D strains (high inducer). Quantitative Trait Loci (QTL) analysis implicates ROCTR candidates are encoded on T. gondii chromosomes X and XII, and appear to be interacting as revealed by a 2-dimensional genome scan. A third ROCTR exists on chromosome VIIb, which controls the early differentiation of IFNγ+ CD8 T cells, as revealed by a T57 x GREAT mouse line that reports IFNγ transcript levels by YFP and flow cytometry. Currently we are investigating candidates on chromosomes VIIb and XII, as these loci produced the highest LOD scores in the QTL analysis. Identifying ROCTR and its mechanism of action can help support the development of vaccines or treatments that aid the immune system to effectively eliminate T. gondii during an infection. Furthermore, identifying ROCTR may yield clues to the selective niches, imposed by immune pressure, that drive T. gondii strain diversity in nature.

Presenter: Romero, Alicia

Dietary iron-induced lipolysis reduces colitis severity during Citrobacter rodentium infection in C57BL/6 mice

Alicia Romero, Janelle Ayres

UCSD/Salk Institute

Iron is an essential nutrient for both host and pathogen. During infection, hosts employ an ensemble of resistance defenses that ultimately limit a pathogenâ□□s access to ironâ□□ a process referred to as nutritional immunity. Likewise, pathogens have evolved a plethora of counter strategies to overcome nutritional immunity and acquire iron within their hosts. Altogether, the principle of nutritional immunity holds that during infection, a surplus of iron will promote pathogen proliferation and decrease host health and survival. And while nutritional immunity thoroughly describes the consequence of excess iron during infection, it does not address how excess iron influences host physiology, and how these changes may independently affect infection dynamics. Our lab has recently described one such model where C3H/hEJ mice fed an iron rich diet become transiently insulin resistant and exhibit increased glucose availability in the gut. This iron-dependent physiological response leads to attenuation of Citrobacter rodentiumâ□□thus, demonstrating that nutritional supplementation can give rise to physiological responses that dramatically affect infection dynamics. However, this model does not extend to other strains of mice, highlighting that genetic background profoundly influences physiological responses to nutrient supplementation. Here, we describe an infection model where the genetically distinct murine strain, C57BL/6, challenged with C. rodentium exhibit increased resistance defenses when fed an iron rich diet. Interestingly, increased resistance does not confer an increase in host healthâ□□owing to the independent effects of excess iron on host physiology. This study examines the effects of dietary iron on C57BL/6 physiology, and how these physiological shifts enhance host resistance defenses during C. rodentium infection.

Presenter: Roncaioli, JL*

NAIP-NLRC4 deficient mice are a new model of Shigella Pathogenesis

JL Roncaioli, Mitchell PS*, Rauch I, Chavez RA, Lee AY, Bergen I, Vance RE*

UC Berkeley

Shigella flexneri is a human-specific bacterial pathogen that invades and replicates within the colonic and rectal epithelium, causing severe and often bloody diarrhea which claims >200,000 lives each year. A challenge in studying and combating Shigella-induced disease is the lack of genetically tractable in vivo models that accurately reflect human infection. It is not clear why mice are resistant to Shigellosis, but we hypothesized that protection might be mediated by the NAIP-NLRC4 inflammasome, a cytosolic innate immune sensor that recognizes components of Shigellaâ□□s type three secretion system (T3SS). We have found that mice pre-treated with oral streptomycin and deficient for the NAIP-NLRC4 inflammasome are acutely susceptible to oral Shigella challenge and display hallmarks of human Shigellosis. Among these, we observe weight loss, malaise, diarrhea, and blood and neutrophils in the stool. Upon histological examination, infected NAIP-NLRC4 knockout mice exhibit significant edema, epithelial damage, hyperplasia, and inflammatory infiltrate in both the cecum and the colon relative to infected wild-type mice. Inflammation depends on both T3SS-dependent epithelial cell invasion and IcsA-dependent cell-to-cell spread in the absence of NAIP-NLRC4. Furthermore, mice that express NAIP-NLRC4 only in the intestinal epithelium phenocopy wild-type mice, suggesting that activation of the inflammasome specifically in the epithelium is sufficient to protect against epithelial cell invasion, spread, and resulting disease. Our findings provide the first oral infection mouse model of Shigella. Our work highlights the importance of epithelial cell inflammasomes in defense against enteric pathogens and provides insight into the species-specific differences that control resistance to Shigella infection.

Presenter: Rozich, Isaiah

Uncovering the molecular mechanisms of cytokine synergy in Natural Killer cells

Isaiah Rozich, Kelly Hudspeth, Sadie Signorella, Han-Yu Shih, Fred P. Davis, John J. Oâ□□Shea

National Institutes of Health

Cytokines act cooperatively to provoke unique phenotypes unachievable when they act alone. For example, stimulating Natural Killer (NK) cells with both IL-12 and IL-18 massively induces IFN-?, whereas either IL-12 or IL-18 alone only slightly induces this important effector cytokine. While the effects of cytokine synergy on IFN-? expression have been explored, questions regarding its global effects and molecular mechanisms remain largely unanswered. Here, we measured transcriptome-wide gene expression (RNA-seq) to globally characterize the transcriptional response of NK cells to IL-12 and IL-18. It was revealed that this cytokine pair synergistically induces the expression of very few genes. Currently, we are exploring the molecular mechanisms that explain how IL-12+IL-18 synergistically induce gene expression by measuring transcription factor binding (ChIP-seq). We have three working hypotheses. Near the transcription start site of each synergistically induced gene, there is (1) increased transcription factor binding magnitude, (2) decreased distance between transcription factor binding peaks, and/or (3) increased number of transcription factor binding peaks. A deeper understanding of NK cell activation mechanisms could improve existing immunotherapies used to treat cancer.

Presenter: SÃ¡nchez-Arcila, Juan Camilo

Using the Collaborative Cross to study adaptive immunity to Toxoplasma gondii

Juan Camilo SÃ¡nchez-Arcila, Darian Galvez, Jennifer Eggleston, Scott P Souza, Kirk DC Jensen

Department of Molecular and Cell Biology, University of California Merced

Toxoplasma gondii, the causative agent of human toxoplasmosis, is distributed worldwide and infects billions of people. Similar to other parasitic infections, immunological responses induced by T. gondii are not sterilizing, leading to the possibility of multiple re-infections during oneâ□□s lifetime. Additionally, there are no fully protective vaccines for any human parasitic pathogen. In this project, we employ an unbiased genetic screen to find novel immunological responses required to protect against a highly virulent strain of T. gondii. The Collaborative Cross (CC) is a panel of multi-parental recombinant inbred mouse lines derived from eight founders of laboratory and wild-derived origin that span the three major Mus musculus subspecies (M. domesticus, M. musculus, M. castaneus). The CC panel captures ~90% of the genetic variation within the Mus musculus species, affording discovery of novel biological mechanisms. To determine new requirements for immunity to T. gondii, the immune responses of 53 CC lines are currently being interrogated. Our model consists of challenging mice with a highly virulent French Guyana strain, GUY-DOS, capable of evading immunological memory responses generated in some but not all founder CC lines. Quantitative Trait Locus (QTL) analysis of loci associated with susceptibility to low virulent natural infections, weight loss (as a marker of pathogenicity), antibody reactivity to T. gondii, and survival to GUY-DOS secondary infections in vaccinated or naturally infected animals are being mapped. In a more advanced stage of the project, we will also perform gene expression-QTL analysis to map variables obtained after transcriptomic analysis of the studied mice. We hypothesize that the CC system will reveal new immunological insights that will aid in vaccine design for T. gondii. Ongoing results from this project will be discussed.

Presenter: Sandstrom, Andrew

A Common Mechanism of NLRP1B Inflammasome Activation by Two Distinct Bacterial Effectors

Andrew Sandstrom, Patrick S. Mitchell, Lisa Goers, Edward W. Mu, Cammie F. Lesser, Russell E. Vance

University of California Berkeley

Inflammasomes are large multi-protein complexes that respond to a range of stimuli to recruit and activate Caspase-1 (CASP1). Activation of CASP1 leads to the maturation and release of pro-inflammatory cytokines and pyroptosis, a lytic form of cell death. Some inflammasomes are activated through the recognition and binding of pathogen-associated ligands. However, others respond to pathogen-associated activities, such as potassium efflux or the enzymatic activity of pathogen-secreted effectors. Yet, the molecular mechanisms by which pathogen-associated activities lead to inflammasome activation have remained unclear. To uncover how inflammasomes respond to pathogen-associated activities we investigated how the NLRP1B inflammasome senses pathogen-associated protease activity. NLRP1B is activated after proteolytic cleavage by the Lethal Factor (LF) protease secreted by Bacillus anthracis. In a mechanism we term â□□functional degradationâ□□ we found that cleavage of NLRP1B by LF leads to the proteasomal degradation of NLRP1B. Degradation liberates a processed C-terminal fragment of NLRP1. This C-terminal fragment then oligomerizes to recruit and activate CASP1. This model of activation further led us to identify IpaH7.8, an E3 ligase secreted by Shigella flexneri, as a novel activator of NLRP1. These results provide insight into how a single protein can sense and respond to diverse pathogen-associated activities.

Presenter: Savage, Hannah

Development of Faux-Biotics to prevent and manage antibiotic-resistant infections

Hannah Savage, Hannah P. Savage, Erin E. Olsan, Eric M. Velazquez, Stephanie A. Cevallos, Henry Nguyen, and Andreas BÃ¤umler

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Antibiotic-resistant bacterial infections are a significant concern for both human and animal health, and the occurrence of antibiotic-resistant infections is expected to continue to rise. After a single antibiotic dose, a fecal microbiota transplant (FMT) from a healthy donor provides colonization resistance against antibiotic-resistant infections. However, in the face on ongoing antibiotic treatment, Faux-Biotics, or non-biotic replacements, for FMTs are needed to confer protection. Using E. coli KPC, a carbapenem-resistant Enterobacteriaceae (CRE), we have found that the combination of Clostridia and endogenous Enterobacteriaceae can replicate the protective ability of a full FMT in mice. Secretion of butyrate by Clostridia alters host epithelial cell metabolism to reduce oxygen and nitrate in the intestinal lumen. This function can be replicated by the drug 5-aminosalicylic acid, which provides full protection against CRE in combination with Enterobacteriaceae or partial protection when used alone. We hypothesize that endogenous Enterobacteriaceae contribute to protection by directly competing with CRE for electron acceptors. Investigations into replacements for endogenous Enterobacteriaceae are ongoing. Further studies of the mechanisms behind colonization resistance will provide insights into potential prevention and treatment options for patients on antibiotics to protect against antibiotic-resistant infections.

Presenter: Serwas, Nina

A novel endocytic mechanism used for antigen transfer from peripheral to immune cells

Nina Serwas, Kyle Marchuk, Rulan Yi, John Ngo, Andrew Oberst, Matthew Krummel

UCSF

Beside protecting an organism from dangerous â□□foreignâ□□ pathogens, one of the most crucial roles of the immune system is to tolerate â□□selfâ□□ and prevent the development of autoimmunity. Central tolerance mechanisms during cell development prevent outgrowth of autoreactive lymphocytes. However, a significant number of autoreactive cells escape these mechanisms, thus, peripheral systems developed to keep immune responses under control. These include the presentation of â□□selfâ□□ by specialized antigen presenting cells (APCs) in a non-inflammatory context. It is not fully understood how APCs endocytose self-antigen and how they are able to distinguish non-harmful â□□selfâ□□-antigen from potential dangerous â□□foreignâ□□. To elucidate this mechanism, we developed a co-culture system of immune cells with malignant or primary cells which have been modified to constitutively express cytoplasmic ZsGreen, a highly stable fluorescent protein. We applied high resolution lattice light sheet live microscopy (LLSM) and flow cytometry to understand how antigen from the cytosol of these cells is transferred to immune cells and how this antigen then is intracellularly sorted after uptake. Our data reveals that immune cells acquire antigen from various malignant and primary cells in a contact-dependent manner. With LLSM we were able to capture the actual moment of transfer. Self-antigens from living cells are transferred to immune cells in numerous small particles (1-5 μm). These packages of antigen are actively nibbled off from protrusions of the antigen-providing cells. The process is dependent on actin polymerization and the activity of different classes of phosphoinositide 3-kinases. Importantly, the transfer is clearly distinguishable from uptake of particles from cells undergoing programmed cell death which represent potential harmful antigens. Those particles are larger in size and separate from dying cells also in the absence of immune cells. Once separated, particles bind to the surface of immune cells until they are eventually invaginated. In summary, we provide detailed insides in how antigens from live cells enter into cells of the immune system and discriminate this mechanism from uptake of dying cells. Future work will address how perturbation of this antigen trading will affect immune regulation and provide a potential future therapeutic angle for modulations of immune tolerance.

Presenter: Shallberg, Lindsey

Contribution of the transcription factor Nr4a1 to CD8+ T cell activation following vaccination

Lindsey Shallberg, Anthony T. Phan, David A. Christian, Christopher A. Hunter

University of Pennsylvania, Department of Pathobiology

CD8+ T cells are critical for resistance to a broad range of pathogens. Presentation of cognate antigen to naive CD8+ T cells results in their activation and expansion, and re-encounter of antigen induces cytokine production, cytolysis of infected cells, and can influence fate decisions. The transcription factor Nr4a1 is an immediate-early response gene to TCR stimulation. It has been reported that Nr4a1 expression is a driver of T cell exhaustion following LCMV-clone13 infection, however it is unclear what impact Nr4a1 expression has in CD8+ T cells that are stimulated by antigen but do not become exhausted. Utilizing vaccination with a non-replicating strain of the OVA expressing intracellular parasite Toxoplasma gondii, we find Nr4a1 expressing OT-I following vaccination. Using a Nr4a1 shRNA knockdown retrovirus, OT-I deficient in Nr4a1 fail to properly expand following vaccination. This is associated with decreased expression of the IL-2 receptor and effector markers such as CX3CR1, as well as a decrease in the proportion of CD62LlowCD127low effector cells. Further, loss of Nr4a1 also altered OT-I mitochondrial mass and function, indicating a role for Nr4a1 in coordinating the metabolic activity necessary to sustain T cell activation and expansion. These data suggest a role of Nr4a1 in generating the protective CD8+ T cell response following vaccination.

Presenter: Shenoy, Meera

Mechanisms of Maternal Suppression of Neonatal Anti-Commensal Immune Responses

Meera Shenoy, Meghan Koch

Fred Hutchinson Cancer Research Center

To ensure host health, the immune system must be able to discriminate between pathogenic and beneficial microbes, but potential pathogens and commensals can share many of the same traits, making this an incredibly difficult task. This process is further complicated within early life, when the immune system is still developing and is first encountering microbes. These early interactions may play a fundamental role in shaping the immune systemâ□□s ability to distinguish friend from foe. As the majority of early-life microbes are provided to the neonate by the mother, we hypothesize that microbial and immune signals passed from the mother to the neonate following birth serve to educate the neonatal immune system. Recent work from our group demonstrated that breast milk derived T-independent maternal antibodies suppress the formation of intestinal T-dependent antibody responses in the neonate. Using mouse models lacking specific immunoglobulin isotypes, we demonstrate that maternal IgG2b and IgG3 are necessary for suppressing neonatal immune responses. These maternal antibodies are required post-birth up to day 14 of life in order to prevent T-dependent antibody responses, and in their absence, there is significant accumulation of commensal microbes within the mesenteric lymph nodes. Mice lacking maternal antibodies demonstrate no obvious defects in global barrier function as measured by translocation of antigens they have not previously experienced. Based on these results, we propose that maternal IgG is suppressing neonatal antibody responses through Fc receptors in an antigen-specific manner. As such, we have obtained mouse models deficient in activating and inhibitory Fc receptors to investigate whether these receptors mediate maternal IgG effects. Through these studies, we are establishing the factors which prevent the development of anti-commensal antibody responses, which is the first step in being able to predict and prevent the development of anti-commensal associated chronic diseases such as inflammatory bowel disease.

Presenter: Shobaki1,2, Nour

Reversing the pro-tumorous functions of tumor-associated macrophages using siRNA-loaded lipid nanoparticles

Nour Shobaki1,2, Yusuke Sato1, Hideyoshi Harashima1

Laboratory for Molecular Design of Pharmaceutics, Hokkaido University, Japan 1, Fred Hutchinson Cancer Research Center, Seattle, WA 2

Tumor-associated macrophages (TAMs) are highly present in the tumor-microenvironment (TME), they generally have pro-tumorous functions and enhance tumor survival and progression [1,2]. Targeting TAMs to reprogram their phenotype and function via siRNA-based gene silencing could be a promising approach for cancer immunotherapy. In this study, a lipid nanoparticle (LNP) formulation composed primarily of an original and a pH-sensitive cationic lipid (CL4H6 lipid), referred to as CL4H6-LNP [3], was optimized and used to target and deliver siRNA to TAMs. The CL4H6-LNP induced strong gene silencing in murine bone marrow-derived macrophages (BMDM) with a superior efficiency compared to the Invitrogenâ□¢ Lipofectamineâ□¢ RNAiMAX transfection reagent, measured by qRT-PCR at the mRNA level of the model target gene (CD45). The CL4H6-LNP was optimized for high stability in the blood circulation, and it induced high and selective uptake and gene silencing in TAMs in BALB/c Ajcl-nu/nu mice carrying human tumor xenograft ((OS-RC-2); renal cell carcinoma (RCC)). After intravenous administration of the LNPs at two doses of 2 mg siRNA/kg, they induced ~70% gene silencing of the model target gene (CD45) at the protein level, measured by flow cytometry. Furthermore, an anti-tumor therapeutic response was obtained by targeting TAMs using the siRNA-loaded CL4H6-LNPs. The anti-tumor therapeutic response was obtained through the silencing of two targeted genes responsible for the pro-tumorous functions of TAMs at three doses of 1 mg siRNA/kg. The treatment increased macrophage (CD11b+ cells) infiltration to TME by 59% and increased the proportion of CD169+ cells by 50%, compared to PBS(-)-treated mice as measured by qRT-PCR. The treatment also reversed the pro-tumorous functions of TAMs, mainly angiogenesis and tumor cell activation, evaluated by the decrease of expression of CD31, vascular endothelial growth factor receptor 2 (VEGFR2), and transforming growth factor β (TGF-β) by 37%, 12%, and 43%, respectively. This research has promising clinical and pharmaceutical applications as novel macrophage-based cancer immunotherapies for human patients. [1] Komohara Y., et al., Adv. Drug Deliv. Rev., 99(Pt B),180-185 (2016) [2] Li X., et al., Mol. Cancer, 18, 177 (2019) [3] Sato Y., et al., J. Control. Release, 295, 140-52 (2019)

Presenter: Soveg, Frank W.

Membrane-targeting of oligoadenylate synthetase 1 primes antiviral activity

Frank W. Soveg, Johannes Schwerk, Katharina Esser-Nobis, Alison Kell, Adriana Forero, Julian Smith, Justin Roby, Tien-Ying Hsiang, Amy Stone, Chiraag Balu, Jonathan Clingan, Daniel B. Stetson, Michael Gale Jr., and Ram Savan

The University of Washington

Positive-strand RNA viruses, such as flaviviruses and picornaviruses, replicate within a modified subcellular compartment known as the replication organelle (RO). Derived from organelle membranes, the RO sequesters viral RNA (vRNA) synthesis from immune surveillance. Although many important vRNA sensors are cytosolic and have limited access to the RO, we hypothesized membrane-targeting of host sensors is a mechanism to gain access to the RO. We tested this hypothesis in the context of oligoadenylate synthetase (OAS) proteins, a family of innate vRNA sensors critical for defense against RNA viruses through activation of the latent endoribonuclease RNase L. Human OAS1 is C-terminally spliced into several isoforms and previous genetic studies have implicated a splice acceptor site SNP (A/G, rs10774671) in the OAS1 gene with West Nile virus (WNV) resistance. The resistance allele (G) controls p46 expression while the susceptibility allele (A) controls p42 expression, but how this SNP promotes resistance to WNV is unknown. We show, compared to the p42 isoform, p46 has significantly stronger antiviral activity against not only WNV, but also other positive-strand RNA viruses such as encephalomyocarditis virus (EMCV) and Coxsackievirus B3 (CVB). Although both OAS1 isoforms require catalytic activity and RNase L for their antiviral activity, only the p46 isoform contains a C-terminal prenylation motif. The addition of a hydrophobic lipid through prenylation facilitates membrane targeting. We tested if membrane-targeting drives the enhanced antiviral activity of p46 using confocal microscopy, biochemical, and genetic approaches. We found prenylation-dependent localization of p46 to the Golgi, while p42 is cytosolic. Through microscopy and RNA immunoprecipitation experiments, we revealed the p46 isoform has enhanced access to vRNA. Collectively, our study shows membrane-targeting of p46 through prenylation localizes this protein in proximity to pools of vRNA generated by positive-strand RNA viruses and provides a mechanistic explanation for the resistance to WNV conferred by the G allele of rs10774671. Broadly, these data show the subcellular positioning of innate immune sensors is important for proper detection of viral nucleic acids.

Presenter: Stull-Lane, Annica

Non-typhoidal Salmonella that causes invasive disease evades reactive oxygen species production by neutrophils

Annica Stull-Lane, Hirotaka Hiyoshi, Vladimir Diaz-Ochoa, RenÃ©e Tsolis

University of California, Davis

Neutrophils are important phagocytic innate immune cells that help combat non-typhoidal Salmonella (NTS) disease, from gastroenteritis to systemic infection. ST19 NTS isolates are associated with classic gastroenteritis, whereas ST313 NTS isolates are more likely to cause invasive disease globally. Invasive NTS disease has a case fatality rate of 20-25% and disproportionately affects the immunologically vulnerable. As part of their antimicrobial arsenal, neutrophils can eliminate microbial pathogens with a potent oxidative burst of reactive oxygen species (ROS). In this study we characterize induction of ROS by ST19 and ST313 strains in both mouse neutrophils ex vivo and in human HL-60 cells differentiated into neutrophil-like cells in vitro. ROS is detected by luminol-based chemiluminescence. Further, we assess the potential for intracellular survival of these strains within the neutrophil with a gentamicin protection assay. Interestingly, ST313 strains induce significantly less ROS than ST19 strains in both mouse and human neutrophils. Without potent ROS induction, levels of intracellular ST313 increase over the course of 2 hours, whereas levels of ST19 do not. These results suggest that invasive disease isolates may use neutrophils as a temporary intracellular niche. Unraveling these host-microbe mechanisms will deliver important fundamental knowledge to the pathogenesis of virulent Salmonella strains, informing future research and therapeutics to combat invasive disease.

Presenter: Tomala, Jakub

Cytokine-antibody single-chain fusions for cancer immunotherapy

Jakub Tomala, E. Leonard, S. Ludwig, H. Yang, M. Leff, J. Spangler and M. Kovar

Institute of Microbiology of Academy of Sciences of the Czech Republic, Prague, Czech Republic

Interleukin-2 (IL-2) is a multifunctional cytokine that is able to potently stimulate immune effector cells (e.g., CD8+ T and NK cells). Unfortunately, its concurrent promotion of regulatory T cells (Treg) and harmful off-target effects have limited its clinical efficacy. Boyman et al. (1) reveal methods with which to mitigate these issues by complexing mouse IL-2 to anti-IL-2 mAb S4B6. These IL-2 complexes are superior to free IL-2, they manifest selective stimulatory activity for memory CD8+ T and NK cells and possess significant antitumor activity. However, the potential clinical use of these complexes is limited due to the mouse origin of IL-2 and the dissociation of the complexes at low concentrations. Based on our previous studies, we designed, engineered and produced translationally relevant protein chimera (immunocytokine, IC) consisting of hIL-2 linked through a flexible oligopeptide spacer to light chain of anti-hIL-2 mAb MAB602, either in unmodified or mutated version, functionally similar to scIL-2/S4B6 immunocytokine (2). This approach circumvent disadvantages of IL-2/S4B6 mAb complexes and exerts sufficient biological activity. We demonstrate that this IC we produced contained both IL-2 and mAb in a single molecule and IL-2 interacted with binding site of mAb. We also demonstrate its biophysical characteristics related to IL-2 receptor and its biological activity in vitro and in vivo. 1. O. Boyman, M. Kovar, M. P. Rubinstein, C. D. Surh, J. Sprent, Selective stimulation of T cell subsets with antibody-cytokine immune complexes. Science 311, 1924-1927 (2006). 2. J. Tomala et al., Chimera of IL-2 linked to light chain of anti-IL-2 mAb mimics IL-2/anti-IL-2 mAb complexes both structurally and functionally. ACS Chem Biol 8, 871-876 (2013).

Presenter: Townsend, Michelle

MOTO-CARsâ□¢: Driving Immunotherapy Forward

Michelle Townsend, Kelsey A. Bennion, Guoying Wang, Zachary D. Ewell, David Lum, Michael Boyer, and Kim L. Oâ□□Neill

Brigham Young University

Immunotherapy is becoming a new standard for cancer treatment. By engineering patient immune cells to target tumors, researchers are harnessing the immune system to combat cancer. Utilizing vectors designed to introduce an scFv fused to a signaling domain, these engineered cells have the ability to specifically identify and target cancer cells. While a majority of the field is focused on T-cell chimeric antigen receptors (CAR T-Cells), these cells have several inherent problems within solid tumors, as they are unable to traffic to the tumor site effectively. We investigated the use of an engineered macrophage toll-like receptor chimeric antigen receptor (MOTO-CARâ□¢) designed to target mesothelin. We found that upon exposure to mesothelin-positive tumor cells (HCC-1806), murine MOTO-CARsâ□¢ are not only effective at eliminating the cancer cells, but also secrete significant levels of TNF-a when compared to mock controls (p-value = 0.0093), indicating activation and polarization to an M1 phenotype. When MOTO-CARsâ□¢ are co-incubated with cancer cells both in vitro and in vivo there is a significant decrease in tumor growth (p-value < 0.0001 in vitro; p-value = 0.0006 in vivo). In NSG mouse models, the tail vein injected MOTO-CARsâ□¢ were able to traffic to the tumor and elicit an anti-tumor response. These engineered cells are most effective within the first 48 hours post transfection. Furthermore, we found that MOTO-CARsâ□¢ are stably transfected for over seven days. Our results show that macrophages are a viable alternative to CAR T-cells and can be engineered to specifically target cancer cells via a cancer-specific scFv. We anticipate increased MOTO-CARâ□¢ efficacy within an immunocompetent mouse model as the secreted TNF-a will aid in polarizing the tumor microenvironment towards an inflammatory, anti-tumor state. Additionally, the MOTO-CARsâ□¢ will present cancer-specific antigen to the host adaptive immune response to elicit a cancer-specific response as opposed to a target-specific response.

Presenter: Trivedi, Shubhanshi

Phenotype and function of mucosal-associated invariant T (MAIT) cells in experimental and clinical sepsis.

*Shubhanshi Trivedi, Daniel Labuz *, Cole Anderson *, Claudia Araujo^, Toni Blair^, Elizabeth Middleton$, Alex Tran #, Matthew A. Mulvey #, Robert A. Campbell^, J. Scott Hale#, Matthew T. Rondina &,^, Daniel T. Leung *,#*

*Division of Infectious Disease, #Division of Microbiology and Immunology, $Division of Pulmonary and Critical Care, University of Utah, School of Medicine, &George E. Wahlen VAMC Department of Internal Medicine and GRECC, Salt Lake City, Utah, ^Molecular Medicine Program, University of Utah, Salt Lake City, Utah

Sepsis is an acute systemic inflammatory response to infection associated with high morbidity and mortality. Mucosal-associated invariant T (MAIT) cells are innate-like T cells enriched in mucosal tissues that recognize bacterial ligands and are activated during infections. Here, we investigated the function and phenotype of MAIT cells during clinical and experimental sepsis, and the mechanisms by which MAIT cells either contribute to or protect against sepsis pathology. We found that, compared to age- and sex-matched healthy donors and paired 3-month convalescence samples, MAIT cells in acutely septic patients were significantly reduced in frequency and displayed an increase in activation markers. When stimulated in vitro, MAIT cells from acutely septic patients had decreased IFN-γ production, changes which are reversed at convalescence. In a murine model of sepsis, MAIT cells expressed lower levels of IFN-γ and IL-17α, whereas these changes were not seen in non-MAIT T cells. Finally, MAIT-deficient (MR1-/-) mice had significantly increased sepsis-induced mortality and bacterial load compared to wild type, associated with reduced tissue-specific cytokine responses. Our data suggest that MAIT cells are highly activated and dysfunctional during clinical and experimental sepsis, and that MAIT cells may contribute to tissue-specific cytokine responses that are protective against mortality due to acute sepsis.

Presenter: Trivedi, Shubhanshi

Increased susceptibility to Klebsiella pneumonia and impaired lung innate immunity after Salmonella Typhimurium intestinal infection.

Shubhanshi Trivedi, Allie Grossmann2, Owen Jensen1 and Daniel T. Leung1,3.

1Division of Infectious Diseases, 2Division of Anatomic Pathology, 3Division of Microbiology and Immunology, University of Utah, Salt Lake City, UT, USA.

Pneumonia and diarrhea are the two leading causes of death in children under age 5 worldwide. Epidemiologic studies in low-resource settings have suggested that a prior episode of diarrhea is a direct risk factor for subsequent respiratory infection. Our aim was to determine the impact of intestinal infection on innate immune responses in the lung and the mechanisms behind lung-gut immunological crosstalk. Using a mouse model of intestinal infection with Salmonella enterica serovar Typhimurium (S. Typhimurium), we investigated how infection in the gut compartment can modulate immunity in the lungs and impact susceptibility to respiratory bacterial (Klebsiella pneumoniae) challenge. Using flow cytometry, we found higher frequencies of plasmacytoid dendritic cells (pDCs) and lower frequencies of monocytic dendritic cells (moDCs) in lungs of S. Typhimurium infected mice compared to uninfected mice. Furthermore, on subsequent challenge with Klebsiella pneumoniae, we found that mice with prior intestinal infection have higher lung bacterial burden and responded with lower frequencies of lung moDCs and neutrophils compared to mice without prior intestinal infection. Hematoxylin and eosin staining demonstrated increased microthrombosis, intravascular polymorphonuclear neutrophils and margination in lungs of mice with prior intestinal infection compared to mice without prior intestinal infection. In conclusion, this study reveals potential role of neutrophils and dendritic cells in immunological crosstalk between the lung and the gut during enteric infections that may affect susceptibility to subsequent respiratory infection. Our findings have the potential to uncover novel therapeutic strategies targeting the gut-lung axis during intestinal infections.

Presenter: Uhrlaub, Jennifer

Chronic Toxoplasma gondii infection blunts the immune response to West Nile virus increasing susceptibility

Jennifer Uhrlaub, Jennifer L. Uhrlaub, Kathryn E. McGovern, Anita A. Koshy, and Janko Nikolich-Å½ugich

University of Arizona

Toxoplasma gondii (T. gondii), a common brain-tropic parasite, is estimated to chronically infect the central nervous system (CNS) of up to a third of the worldâ□□s population. A continuous immune response prevents the reactivation of cysts within the CNS and dramatically alters the immune landscape of the brain. Whether, and to what extent, the presence of both the parasite and the immune cells that keep it in check impact the immune response to other neurotropic infections has not been well studied. West Nile virus (WNV) is a mosquito-borne infection with a clinical spectrum ranging from asymptomatic or mild flu-like symptoms to more severe neuroinvasive conditions, including meningitis, encephalitis, acute flaccid paralysis, and death. Leveraging well characterized mouse models for both T. gondii and West Nile virus (WNV), we assessed the impact of chronic T. gondii infection on the immune response and outcome to WNV. We determined that granzyme effector function is reduced in WNV-specific CD8 T cells and susceptibility to WNV is increased; predominantly in female mice.

Presenter: Valencia, Clint

Determining IL-7/IL-7R function in fetal macrophage development

Clint Valencia, Gabriel Leung, Anna Beaudin
University of California, Merced

Little is known regarding the mechanisms or factors that play a part in the development of specialized macrophages during fetal development. Our recent work has revealed the interleukin-7 receptor (IL-7R) as an important regulator of fetal macrophage development. Germline deletion of IL-7R or pharmacological blockade during development impairs macrophage development in the liver, lung, and epidermis. We now aim to delineate how IL-7R regulates tissue resident macrophage development together with its cognate cytokine, IL-7. In adult hematopoiesis, IL-7 is a critical cytokine responsible for the proliferation and survival of lymphocytes, including T cells. While the role of IL-7/IL-7R signaling in promoting the lymphoid lineage is well-established, little is known regarding the role of IL-7/IL-7R signaling in myeloid cell development. To gain insight into how IL-7/IL-7R signaling is regulating myeloid development, we investigated IL-7 expression in fetal tissues and the spatial relationship between IL-7R-expressing macrophages and IL-7-expressing cells in distinct fetal tissues. Preliminary analysis suggests that clusters of IL-7R expressing macrophages lie adjacent to cells that express IL-7 within fetal tissues. We also investigated tissue macrophage cellularity in IL-7 KO mice, and found that germline deletion of IL-7 also significantly impaired tissue macrophage development in the brain, liver, and epidermis of neonates. Together with ongoing experiments to define how IL-7 regulates tissue macrophage development, these findings help elucidate developmental mechanisms underlying the establishment of unique populations of tissue-resident macrophages across ontogeny.

Presenter: Van Dis, Erik

Interferon gamma independent control of Mycobacterium tuberculosis by CD4 T cells

Erik Van Dis, Kimberly M Sogi, Chris S Rae, Kelsey E Sivick, Janet Peace Babirye, Lily H McCann, Sarah M McWhirter, Daniel A Portnoy, and Sarah A Stanley

Univ. of California, Berkeley

IFN-γ produced by CD4 T cells is crucial for controlling Mycobacterium tuberculosis (Mtb). However, results from both human and mouse studies suggest that production of IFN-γ is not sufficient for control of infection. Here, we show that a protein subunit vaccine formulated with STING-activating cyclic dinucleotides elicits durable protective immunity to Mtb in the mouse model, and that this protection correlates not with IFN-γ-producing Th1 T cells but with a robust Mtb-specific Th17 response. This vaccine provides protection that is superior and more durable than that of the live attenuated vaccine strain BCG, and full protective efficacy is IL-17 dependent. These results and other published mouse studies suggest that IFN-γ-independent CD4 T cell mechanisms contribute to control of Mtb, but how CD4 T cells exert a protective effect independent of IFN-γ remains unknown. Using an ex vivo co-culture system we show that CD4 T cells isolated from the lungs of Mtb-infected mice activate macrophages to control Mtb replication in an IFN-γ independent manner. Supernatant from cultured lung-derived CD4 T cells is sufficient for IFN-γ-independent control of Mtb. Roles for known CD4 T cell effectors including TNF-α, CD40, CD153 and Type I IFN have been excluded, suggesting that a novel factor produced by CD4 T cells can activate macrophages to control Mtb infection. RNAseq on infected macrophages cultured with IFN-γ deficient CD4 T cells revealed a unique pattern of activation when compared with conventional IFN-γ-dependent activation. These findings further elucidate the role of CD4 T cells during Mtb infection and may help inform vaccine development which has focused primarily on exploiting classic IFN-γ-centric Th1 immunity.

Presenter: Van Winkle, Jacob

Steady-state interferon-stimulated gene expression in the intestinal epithelium depends on interferon lambda and the bacterial microbiome

Jacob Van Winkle, Stefan Peterson, Michael Wheadon, Sanghyun Lee, Megan Baldridge, and Timothy Nice

Oregon Health & Science University

Distinct cellular responses have evolved that are specialized for eliminating pathogens in mucosal surfaces, such as the gastrointestinal tract. In particular, intestinal epithelial cells (IECs) are uniquely responsive to interferon lambda (IFN-λ) and depend on this cytokine for antiviral defense to a greater extent than other types of interferon. IFN-λ signaling protects IECs by inducing expression of antiviral IFN-stimulated genes (ISGs), but it is unclear how this signaling pathway interacts with the intestinal microbiome. We find that depletion of the intestinal microbiome with broad spectrum antibiotics significantly reduces steady-state ISGs in the gastrointestinal tract when assessed by whole-tissue RNA sequencing. Steady-state ISG expression is also significantly reduced in mice with conditional knockout of IFN-λ receptor (IFNLR) in IECs. These data suggest that enteric bacteria stimulate expression of IFN-λ and, subsequently, epithelial ISGs at homeostasis. Strikingly, imaging data reveals distinct pockets of IFNLR-dependent ISGs throughout the small intestinal epithelium. These highly localized ISGs are also dependent on the presence of bacteria and IFNLR expression by IECs. Notably, in addition to localized ISG expression along the length of the gastrointestinal tract, ISG expression is concentrated in the most mature IECs at the tips of individual villi. These novel observations indicate that ISGs are robustly expressed in localized regions of the intestinal epithelium at homeostasis. This robust ISG expression depends on the bacterial microbiome and may preemptively activate antiviral defenses in vulnerable IECs to improve host fitness against enteric viruses.

Presenter: Waer, Christi

Role of CXCR5+ CD8+ T cells in Regulating Autoimmunity

Christi Waer, Kristen Valentine, Genevieve Mullins, Kirk Jensen, Katrina K. Hoyer
University of California Merced

CXCR5+ CD8+ T cells are an emerging population of immune cells that arise during chronic disease. Depending on the disease setting the cells have been termed CD8+ T follicular cells or stem-like progenitor memory cells. How CXCR5+ CD8+ T cells interact to promote or protect during autoimmunity and cancer remains largely unknown. Our lab has previously discovered CXCR5+ CD8+ T cells within autoimmune germinal centers that promote B cell class switching and plasma cell differentiation. Other labs have observed an expansion of CXCR5+ CD8+ T cells in pancreatic, colon, and lung cancers. This population has also been seen in models of infection, such as LCMV, SIV and HIV. CXCR5+ CD8+ T cells are known to express inhibitory markers that may be suggestive of exhaustion phenotypes or helper responses, such as Tim3 and PD-1. Here we propose to explore CXCR5+ CD8+ T cell interactions within tumor and autoimmune settings to delineate the functional state and impact on disease outcomes.

Presenter: Warner, Lindsey

Thymic epithelial miR-155 promotes regulatory T cell development by safeguarding medullary thymic epithelial cell maturation

Lindsey Warner, Jiayi Dong, Ling-Li Lin, Mei-Chi Chen, Ryan O'Connell, and Li-Fan Lu

Division of Biological Sciences, University of California, San Diego; Moores Cancer Center, University of California, San Diego; Center for Microbiome Innovation, University of California, San Diego; Department of Immunology, University of Washington; Department of Pathology, University of Utah.

During thymocyte development, medullary thymic epithelial cells (mTECs) provide an instructive cellular cross-talk important for shaping discrete thymic microenvironments for not only negative selection, but also the generation of regulatory T (Treg) cells. Here, we identify that miR-155, a microRNA whose expression in Tregs is crucial for their development and homeostasis, also contributes to thymic Treg (tTreg) differentiation by promoting mTEC maturation. Mechanistically, we show that RANK signaling induces miR-155 in order to safeguard the thymic medulla via targeting multiple known and previously uncharacterized molecules within the TGFÃ signaling cascade, a pathway known for its role in restricting mTEC development and differentiation. Our work locates a miR-155-TGFÃ axis in the thymic medulla to determine mTEC maturity and, consequently, the quantity of tTregs, underscoring that miR-155 ensures proper tTreg cell development in both cell-intrinsic and -extrinsic manners.

Presenter: Wu, Glendon

Monoallelic and monogenic Tcrb assembly enforced by Vb recombination signal sequences and physiological roles for Tcrb repertoire and allelic exclusion

Glendon Wu, Author Names separated by commas

University of Pennsylvania

Monoallelic expression of antigen receptor (AgR) genes is assumed to be critical for the proper selection and function of T and B lymphocytes. V(D)J recombination of Tcrb, Igh, and Igk loci is regulated such that most cells assemble and express functional AgR genes from one allele (allelic exclusion). The V gene segment recombination signal sequences (RSSs) of Tcrb and Igh loci are weak and have been proposed to mediate monoallelic V-to-DJ recombination. To test the role of Vb RSSs in promoting Tcrb allelic exclusion, we created and studied mice harboring replacements of the DJCb-distal V2 and -proximal V31 RSSs with the stronger 3â□□Db1 RSS. Here, we show a substantial role for weak Vb RSSs in limiting: Vb recombination frequency, biallelic functional Vb-to-DJb recombination and biallelic TCRb expression, and dual TCRb chain expression encoded by a single Tcrb allele. Our data indicate that weak Vb RSSs limit Vb recombination to promote monogenic Tcrb assembly in the time window before feedback inhibition halts Vb recombination. Given that AgR allelic exclusion is most stringently applied to Tcrb and Igh, whose assembly and expression drives cellular proliferation, we hypothesized that mechanisms directing monoallelic induction of RAG double strand breaks (DSBs) evolved at Tcrb and Igh loci in part to suppress DSBs from entering S phase and forming oncogenic AgR translocations. We find that p53-/- mice with V2 and V31 RSS replacements succumb more rapidly to thymic lymphomas than their p53-/- counterparts, which is consistent with our model. As Vb RSS replacement mice exhibit dramatic shifts in the Vb repertoire, we also have begun using them to test relationships between Vb repertoire and host defense.

Presenter: Wu, Ting-Ting

Virus-like Vesicles as a Vaccine Platform for Kaposi Sarcoma-associated Herpesvirus

Ting-Ting Wu, Alex K. Lam, Danyang Gong, Gurpreet Brar, Carissa I. Pardamean, Xi Ma, Ren Sun

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Background Kaposi Sarcoma-associated Herpesvirus (KSHV) is linked to several devastating diseases such as Kaposi sarcoma, primary effusion lymphoma, and multicentric Castleman disease. Both prophylactic and therapeutic KSHV vaccines would be beneficial for those at high risks of being immunocompromised and those living in endemic HIV areas, especially in Africa where resources are limited. Methods Infected cells produce and release membrane vesicles that resemble virions but do not contain a complete set of viral components required for infectivity. KSHV-infected cells produce a large amount of such non-infectious virus like vesicles (VLVs). We hypothesize that these KSHV-VLV will constitute ideal antigens for a vaccine without the safety risk of a live virus. To test this hypothesis, it is critical to separate KSHV-VLV from infectious virions. Our laboratory generated a viral mutant that only produces KSHV-VLVs that lack capsids and viral genomes. This strategy exploits a mutation that blocks capsid maturation to prevent the production of infectious virions but not KSHV-VLVs. We aimed to assess the immunogenicity of KSHV-VLVs. Results In a preliminary immunization study, injections of KSHV-VLV without adjuvants elicited KSHV-specific humoral immunity. The KSHV-VLV immune serum reacted with the surface of cells expressing KSHV lytic proteins. In addition, it neutralized KSHV and inhibited infection of target cells. Currently, we are investigating whether inclusion of adjuvants increases the immunogenicity of KSHV-VLVs. Conclusions KSHV-VLV provides a novel vaccine platform for KSHV. The efficacy and utility of KSHV-VLV as a vaccine can be further improved by incorporating additional antigens to elicit cellular immunity that targets KSHV latent proteins.

Presenter: Yamashiro, Livia

STING controls Herpes Simplex Virus in vivo independent of type I interferon induction

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The Stimulator of Interferon Genes (STING) pathway initiates potent immune responses upon recognition of DNA derived from bacteria, viruses and tumors. To signal, the C-terminal tail (CTT) of STING recruits TBK1, a kinase that phosphorylates serine 365 (S365) in the CTT. Phospho-S365 acts as a docking site for IRF3, a transcription factor that is phosphorylated and activated by TBK1, leading to transcriptional induction of type I interferons (IFNs). IFNs are essential for antiviral immunity and are widely viewed as the primary output of STING signaling in mammals. However, other more evolutionarily ancestral responses, such as induction of NF-?B or autophagy, also occur downstream of STING. The relative importance of the various outputs of STING signaling during in vivo infections is unclear. Here we report that mice harboring a serine 365-to-alanine (S365A) point mutation in STING exhibit normal susceptibility to Mycobacterium tuberculosis infection but, unexpectedly, are resistant to Herpes Simplex Virus (HSV)-1, despite lacking STING-induced type I IFN responses. Likewise, we find Irf3â□□/â□□ mice exhibit resistance to HSV-1. By contrast, resistance to HSV-1 is abolished in mice lacking the STING CTT or TBK1, suggesting that STING protects against HSV-1 upon TBK1 recruitment by the STING CTT, independent of IRF3 or type I IFNs. Interestingly, we find that STING-induced autophagy is a TBK1-dependent IRF3-independent process that is conserved in the STING S365A mice, and autophagy has previously been shown to be required for resistance to HSV-1. We thus propose that autophagy and perhaps other ancestral interferon-independent functions of STING are required for STING-dependent antiviral responses in vivo.

Presenter: Yang, Letitia

Dual TCR co-expression promotes immune response and memory formation during viral infection

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The normal repertoire of T cells includes a subpopulation co-expressing 2 T cell receptor (TCR) clonotypes. We have shown that dual TCR expression promotes thymic development and homeostatic proliferation, potentially via increased recognition of self-ligands. We hypothesize this could affect T cell repertoires and immune responses, as reactivity against self-antigen influences development and persistence of foreign antigen-specific memory. Despite evidence for the effects of dual TCR cells, they have been understudied due to an inability to definitively identify and test cells expressing 2 TCRs. To address this, we developed a novel mouse model linking eGFP (GFP) or tdTomato (RFP) reporters to the TCR? constant region to generate mice with definitive labeling of single- and dual-TCR cells. Flow cytometry identifies ~16% of peripheral T cells in immunologically naive adult mice as expressing dual TCRs, significantly higher than previous estimates of < 10%. Confocal microscopy demonstrates GFP and RFP signals localized to the cell membrane, indicating functional co-expression. We examined dual receptor T cells during immune responses using a model infection with LCMV-Armstrong. Dual TCR expression did not influence recognition of LCMV antigens in mice pre-infection. However, examination of I-Ab:GP66-77 and H2-Db:GP33-41 reactive cells on d28 post-infection revealed significant increases in LCMV-specific dual TCR cells. Furthermore, dual TCRs cells were specifically and significantly increased among antigen-specific CD8+ central memory cells. These data identify a novel potential role for dual TCR-expressing cells in protective immune responses and formation of immune memory.