Presenter: Abdelfattah, Ahmed

Effect of Selected Osteopathic Lymphatic Techniques on Immune System in Healthy Subjects: Randomized Control Trial

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Aim: this study was designed to investigate and compare the efficacy of selected osteopathic lymphatic techniques on the absolute CD4+ count in healthy subjects. Materials and Methods: Forty-five subjects (33 males and 12 female), age varies from 20 to 50 years old. They were allocated to three groups each one has 15 subjects: first one received sternal pump and sternal recoil techniques for 12 sessions, three sessions per week. Second one received thoracic lymphatic pump and splenic pump techniques for 12 sessions, three sessions per week. Third one (control group) didn't receive OMT. Absolute count of CD4 was used to evaluate participants before and after application of the osteopathic techniques. Results: analysis showed significant increase in CD4 count after treatment in the second group also there was no significance in the first and third groups. P-value was = 0.05. Conclusion and discussion: thoracic lymphatic and splenic pump manipulative techniques are effective methods of enhancing the immune system in healthy subjects (TLPT & SPT). Key words: Osteopathy, Lymphatic techniques, Immune system

Presenter: Akilesh, Holly

Chronic TLR7 and TLR9 signaling drives anemia via differentiation of unique hemophagocytes

Holly Akilesh, Akilesh HM, Buechler MB, Duggan JM, Hahn WO, Matta B, Sun X, Gessay G, Whalen E, Mason M, Presnell SR, Elkon KB, Lacy-Hulbert A, Barnes BJ, Pepper M, and Hamerman JA

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Cytopenias are an important clinical problem associated with inflammatory disease and infection. We show that specialized phagocytes that internalize red blood cells develop in TLR7-driven inflammation. TLR7 signaling caused development of inflammatory hemophagocytes (iHPC) that resemble splenic red pulp macrophages, but are a distinct population derived from Ly6Chi monocytes. iHPC were responsible for anemia and thrombocytopenia in TLR7-overexpressing mice, which have a macrophage activation syndrome (MAS)-like disease. IRF5, associated with MAS in human disease, participated in TLR7-driven iHPC differentiation. We also found iHPC during experimental malarial anemia, where they required endosomal TLR and MyD88 signaling for differentiation. Our findings uncover a mechanism by which TLR7 and TLR9 specify monocyte fate, and identify a unique population of phagocytes responsible for anemia and thrombocytopenia associated with inflammation and infection.

Presenter: Alshetaiwi, Hamad

Delineating the Distinct Cellular and Molecular Properties of Myeloid-Derived Suppressor Cells in Breast Cancer using Single Cell RNA Sequencing

Hamad Alshetaiwi(1,2,3), Nicholas Pervolarakis(4,3), Laura L. McIntyre(5), Dennis Ma(1,3), Quy Nguyen(1,3), Kevin Nee(1,3), Jan Rath(1), Katrina Evans(1,2,6), Leona Torosian(7), Anushka Silva(7), Craig Walsh(5), Kai Kessenbrock(1,2,3)

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Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells with potent immune suppressive activity. These cells accumulate in pathological conditions such as cancer. MDSCs in breast cancer promote angiogenesis, tumor invasion and metastasis. In addition, MDSCs regulate immune response by suppressing T cell (CD4/CD8) proliferation and INFγ production. Multiple mechanisms of immune suppression by MDSCs exist. For instance, the production of reactive oxygen and nitrogen species (ROS, NO) and arginase (Arg). MDSCs, normal neutrophil granulocytes, and monocytes are defined by the same flow cytometry markers, CD11b+Gr1+. MDSCs can be further classified into granulocytic MDSC (G-MDSC) and monocytic MDSC (M-MDSC). Here, we use a breast cancer mouse model (MMTV-PyMT) to study the cellular and molecular properties of MDSCs in single cell resolution. To test MDSCs capacity to inhibit immune responses, CD11b+Gr1+ cells from tumor-bearing PyMT mice and control WT mice were sorted by fluorescence-activated cell sorting (FACS) from bone marrow, lung, and spleen, and then subjected to a T cell activation assay in co-culture with T cells. We found that exclusively CD11b+Gr1+ cells from spleen of tumor-bearing PyMT mice significantly suppresses CD4 and CD8 T cell proliferation and INFγ production in CD8 T cells, whereas CD11b+Gr1+ cells from bone marrow and lung showed no effect on T cell activation. These data indicate that CD11b+Gr1+ cells sorted from spleen from tumor bearing mice are MDSCs because of their ability to suppress T cell function. Ongoing experiments utilize single cell RNA sequencing (ScRNAseq) to directly compare breast cancer derived MDSCs to the respective cell populations harvested from normal, non-tumor bearing animals. Results revealed a distinct G-MDSC cluster from normal neutrophils and a distinct M-MDSC cluster from normal monocytes. Our studies may provide crucial insights into the biology of MDSCs, which may ultimately form the basis for novel marker and therapeutic avenues to improve cancer immunotherapy.

Presenter: Anderson, Kristin

Engineering adoptive T cell therapy to co-opt Fas ligand-mediated death signaling in solid tumors

Kristin Anderson, Kristin G. Anderson, Shannon K. Oda*, Breanna M. Bates, Madison G. Burnett, Edison Y. Chiu, Magdalia L. Suarez Gutierrez, Nicolas Garcia, Andrew W. Daman, Philip D. Greenberg*

Fred Hutchinson Cancer Research Center and the University of Washington

Background: Over 20,000 women are diagnosed with ovarian cancer annually, and over half will die within 5 years. This rate has changed little in the last 20 years, highlighting the need for therapy innovation. One especially promising new strategy employs immune T cells engineered to target proteins uniquely overexpressed in tumors, with the potential to limit tumor growth without toxicity to healthy tissues. Mesothelin (Msln) is a rational target for ovarian cancer immunotherapy - it contributes to the malignant and invasive phenotype in these tumors and has limited expression in healthy cells. Methods: Deep transcriptome profiling of whole tumor tissue was used to confirm the expression of similar gene signatures in human cancers and in the preclinical ID8 mouse model, including comparable expression of immunosuppressive pathways. For example, RNA sequencing, flow cytometry and immunohistochemistry analysis revealed consistently high expression of the immunomodulatory protein Fas ligand (FasL). Human/mouse T cells were engineered to express a human/mouse Msln-specific high-affinity T cell receptor (TCRMsln) and tested for cytotoxic activity against human patient-derived or ID8 mouse ovarian cancer cell lines in vitro and in vivo. Results: In a disseminated ID8 tumor model, adoptively transferred TCRMsln T cells preferentially accumulated within established tumors, delayed ovarian tumor growth, and significantly prolonged mouse survival. However, our data also revealed that elements in the tumor microenvironment (TME) limit engineered T cell persistence and anti-cancer activity. We and others previously detected FasL in the tumor vasculature and TME of human and murine ovarian cancers. FasL can induce apoptosis in infiltrating lymphocytes expressing Fas receptor (Fas). To overcome this potential T cell evasion mechanism, we generated a panel of immunomodulatory fusion proteins (IFP) containing the Fas extracellular binding domain fused to a CD28 or 4-1BB co-stimulatory domain, rather than the natural death domain. Relative to T cells modified with only TCRMsln, T cells engineered to express both TCRMsln and a Fas IFP preferentially infiltrate tumors, expand/persist and retain function in the TME of tumor-bearing mice. Moreover, adoptive immunotherapy with IFP+ T cells significantly prolonged survival in tumor-bearing mice, relative to TCRMsln T cells lacking an IFP. Conclusions: Fas/FasL signaling can mediate T cell death, including activation-induced cell death, an apoptotic mechanism responsible for regulating T cell expansion. Thus, tumor cells may upregulate FasL for protection from tumor-infiltrating lymphocytes. As many solid tumors overexpress FasL, IFPs may provide an opportunity to enhance engineered adoptive T cell therapy against many malignancies.

Presenter: Barry, Kevin

A natural killer-dendritic cell axis defines checkpoint therapy-responsive tumor microenvironments.

Kevin Barry, Hsu J, Broz ML, Cueto FJ, Binnewies M, Combes AJ, Nelson AE, Loo K, Kumar R, Rosenblum MD, Alvarado MD, Wolf DM, Bogunovic D, Bhardwaj N, Daud AI, Ha PK, Ryan WR, Pollack JL, Samad B, Asthana S, Chan V, Krummel MF

University of California, San Francisco

Intratumoral stimulatory dendritic cells (SDCs) play an important role in stimulating cytotoxic T cells and driving immune responses against cancer. Understanding the mechanisms that regulate their abundance in the tumor microenvironment (TME) could unveil new therapeutic opportunities. We find that in human melanoma, SDC abundance is associated with intratumoral expression of the gene encoding the cytokine FLT3LG. FLT3LG is predominantly produced by lymphocytes, notably natural killer (NK) cells in mouse and human tumors. NK cells stably form conjugates with SDCs in the mouse TME, and genetic and cellular ablation of NK cells in mice demonstrates their importance in positively regulating SDC abundance in tumor through production of FLT3L. Although anti-PD-1 'checkpoint' immunotherapy for cancer largely targets T cells, we find that NK cell frequency correlates with protective SDCs in human cancers, with patient responsiveness to anti-PD-1 immunotherapy, and with increased overall survival. Our studies reveal that innate immune SDCs and NK cells cluster together as an excellent prognostic tool for T cell-directed immunotherapy and that these innate cells are necessary for enhanced T cell tumor responses, suggesting this axis as a target for new therapies.

Presenter: Batista, Samantha

The role of IL-1α in the response to chronic *Toxoplasma gondii* infection

Samantha Batista, Katherine Still, Carleigh O'Brien, Tajie Harris
University of Virginia

Toxoplasma gondii is an intracellular parasite that causes chronic infections, in which the parasite forms cysts inside neurons in the brain. Chronic infection is asymptomatic in healthy hosts, but immunocompromised individuals experience severe, even fatal, disease in which replicating parasites destroy brain tissue. Constant immune pressure is required to maintain control of the parasite throughout chronic infection, leading to the localized recruitment of immune cells. Thus, *T. gondii* is an excellent model to study neuroinflammation and immune cell recruitment into the CNS. Our lab observes cell death in focal areas of inflammation in the brain, suggesting the possibility of inflammatory molecule release from dying cells. The alarmin IL-1α is expressed in the brain, and is expressed predominantly by brain-resident microglia and infiltrating macrophages, implicating these cell types as potential sources of this cytokine. I have further evidence to suggest that microglia may be the relevant source in this model. Mice lacking IL-1α or its receptor (IL-1R1), but not IL-1β, have defects in monocyte/macrophage infiltration into the brain during chronic infection, as well as increased parasite burden in the brain. This IL-1α-dependent immune response appears to be dependent on pyroptosis, as caspase-1/11-/- mice also display decreased recruitment of myeloid cells into the brain and increased parasite burden. IL-1R1 is expressed largely on brain vasculature, and IL-1R1-/- mice display decreased mRNA expression of the adhesion molecules ICAM-1 and VCAM-1 in the brain. Blockade of the ICAM-1 ligand LFA-1 during chronic infection shows that macrophage recruitment into the brain is partly dependent on this molecule. Together, these data have led me to suggest a model in which during chronic *T. gondii* infection, IL-1α released from microglia in the brain through pyroptosis acts on the brain vasculature to modulate expression of adhesion molecules, facilitating the infiltration of immune cells to sites of infection.

Presenter: Beaudin, Anna

The lymphoid-associated interleukin 7 receptor (IL-7R) regulates tissue resident macrophage development

Anna Beaudin, Gabriel Leung, Taylor McCann, Atesh Worthington, E. Camilla Forsberg
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The discovery of a fetal origin for tissue-resident macrophages (trMacs) has inspired an intense search for the mechanisms underlying their development. Here, we performed in vivo lineage tracing of cells with an expression history of IL-7Ra, a marker exclusively associated with the lymphoid lineage in adult hematopoiesis. Surprisingly, we found that IL7R-Cre labeled fetal-derived, adult trMacs, in the absence of IL7Ra message or protein expression in labeled cells. Labeling was almost complete in some tissues and partial in other organs. The putative progenitors of trMacs, yolk sac (YS) erythromyeloid progenitors (EMPs), did not express IL-7R, and YS hematopoiesis was unperturbed in IL-7R-deficient mice. In contrast, tracking of IL-7Ra message levels, surface expression, and IL7R-Cre-mediated labeling across fetal development revealed dynamic regulation of IL-7Ra mRNA expression and rapid upregulation of IL-7Ra surface protein upon transition from monocyte to macrophage within fetal tissues. Fetal tissue monocyte differentiation ex vivo produced IL-7R+ macrophages, supporting a direct progenitor-progeny relationship. Additionally, blockade of IL-7R function during late gestation specifically impaired the establishment of fetal-derived tissue macrophages in vivo. These data provide evidence for a distinct function of IL-7Ra in fetal myelopoiesis and identify IL-7R as a novel regulator of tissue-resident macrophage development. Ongoing work addresses the specific cellular functions regulated by IL-7 signaling during fetal myeloid development, and the contribution of IL-7R-labeled macrophages to functional heterogeneity within adult tissue resident macrophage compartments.

Presenter: Bennion, Kelsey A.

Stepping into the ring of cancer immunotherapy: HPRT as a therapeutic target

Kelsey A. Bennion, Michelle H. Townsend, Zac E. Ence, Erica Suh, Abi M. Felsted, John E. Lattin, Stephen R. Piccolo, Richard A. Robison, Kim L. O'Neill

Brigham Young University

Although the role of Hypoxanthine-guanine phosphoribosyltransferase (HPRT) as a housekeeping gene is well established, the roles of HPRT in tumor proliferation are less studied. As cancer is the result of uncontrolled cell proliferation and salvage pathway, enzymes like HPRT play an important role in cell proliferation, we chose to investigate the role of HPRT in tumor growth. Previous experiments show differential expression of HPRT within the cancer cell and on the cell surface. These varied findings motivated us to explore this variation and the potential function of HPRT in tumor proliferation. Our study investigates the potential of HPRT as a therapeutic target and the mechanism by which HPRT is upregulated on the surface of cancer cells. PC3 and DU145 prostate cancer cells were used in this study because of their markedly different levels of surface HPRT expression. Flow cytometry, scanning electron microscopy, and confocal microscopy validated the surface localization of HPRT while immunohistochemistry revealed HPRT upregulation within tissue. In all experiments, we observed a significant association between HPRT and the surface of DU145 cells, but found no HPRT presence on the surface of PC3 cells. Following these observations, we performed ADCC experiments to determine the potential of HPRT as a target for antibody-mediated cell cytotoxicity. Compared to cells treated with isotype antibody, cells treated with HPRT antibody experienced significantly higher cell death in DU145 cells (4.5ug, p-value < 0.0001; 6ug, p-value = 0.028; 8ug, p-value = 0.025) while cells treated with the same antibody experienced insignificant cell death in PC3 cells (4.5ug, p-value = 0.33; 6ug, p-value = 0.35; 8ug, p-value = 0.99). Because HPRT showed promise as a therapeutic target, we began to investigate why HPRT is upregulated on the cell surface as this could help screen patients who would respond well to HPRT targeted immunotherapy. We hypothesized that certain mutations in p53 may be responsible for the upregulation of HPRT found within tumors, as p53 has previously been shown to influence salvage enzyme expression and function. We observed that cancer cell lines with elevated HPRT surface expression (SW-620, Raji, HT29, and DU145) also have mutations in p53 that may exhibit gain of function properties (GOF). Additionally, we observed that cancer cells that lack HPRT surface expression (PC3) are p53 null. Although the correlation between p53 gain of function mutations and HPRT surface upregulation is still unfolding and remains preliminary, HPRT surface levels seem to be dependent on alterations to p53. Overall, the findings from this study support HPRT as a therapeutic target and seek to expand current understanding of the underlying mechanism for HPRT upregulation.

Presenter: Bitter , Eliza

HPRT impact on immune regulation effects the microenvironment

Eliza Bitter, Michelle H. Townsend, Dallas Larsen, Claudia M. Tellez Freitas, Stephen R. Piccolo, K. Scott Weber, Richard A. Robison, and Kim L. O'Neill

Brigham Young University

Hypoxanthine-guanine phosphoribosyltransferase (HPRT) is a protein in the DNA salvage pathway that is responsible for converting inosine and guanine derivatives to their monophosphate forms. Previously, it has been shown that HPRT is upregulated within several cancer types. Due to significant elevation of HPRT within a large proportion of patient tumors, we evaluated if HPRT upregulation could impact immune infiltration in the tumor microenvironment. To first investigate the effects of HPRT levels on immune regulation, we evaluated the changes in immune gene expression in over 9000 lung squamous carcinoma patients. Patients were ranked according to HPRT high and low expression and analyzed for correlation against anti-inflammatory, anti-inflammatory and pro-inflammatory, and pro-inflammatory genes. Of the 194 total genes evaluated, 68% were negatively correlated with HPRT elevation (31 of 49 anti-inflammatory, 54 of 67 anti-inflammatory and pro-inflammatory, and 47 of 78 pro-inflammatory). Because HPRT upregulation appeared to have a significant impact on immune gene regulation, we evaluated the effects of elevated HPRT on patient survival to determine whether the change in immune regulation impacted overall patient health and longevity. Within the range of high and low HPRT expression, patient survival was compared between the top 30% and the bottom 30% in different cancer types. Among those in the higher 30% of HPRT expression there was a significant decrease in overall survival, with Uterine Corpus Endometrial Carcinoma and Renal Papillary Cell Carcinoma showing the greatest difference in survival ($p = 0.001$, $p = 0.007$ respectively). We then reasoned that HPRT is preferentially selected for in the tumor microenvironment for its impact on immune regulation and that due to this characteristic of HPRT, potentially there was a decrease in tumor infiltration of immune cell types. Immune cell infiltration was determined across several different cancer types for B cells, CD8+ T cells, CD4+, T cells, macrophages, neutrophils, and dendritic cells. Results from the analysis indicated an overall significant decrease in immune infiltration when HPRT expression is elevated in macrophages showed the least change in infiltration and CD4+ showed the most significant decrease ($p = 0.001$) across all cancer types tested. From our findings, we determined that HPRT significantly affects the ability of immune cell types to infiltrate the tumor. Our findings point to the tendency of tumors to select for HPRT in order to potentially promote tumor survival and its involvement in immune regulation.

Presenter: Bosio, Catharine

Non-Canonical Control of Replication of Intracellular Bacteria via Modulation of Host Cell Metabolism by IFN-gamma

Catharine Bosio, Forrest Jessop, Robert Buntyn, Tara Wherly, Benjamin Schwarz

Rocky Mountain Laboratories/NIAID/NIH

IFN-γ is universally accepted as a required cytokine to control a wide variety of pathogens, including those residing in the intracellular compartment. However, there is evidence that canonical forms of IFN-γ mediated killing, e.g. ROS, RNS, and tryptophan depletion, of intracellular pathogens are not the primary means by which microbial growth is restricted. Here we provide evidence demonstrating that the ability of IFN-γ to differentially restrict replication of attenuated and virulent strains of the intracytoslic pathogen F. tularensis was not due to these canonical pathways, but rather via manipulation of host cell metabolism. Specifically, we show that attenuated strains (Live Vaccine Strain [LVS]) readily killed in IFN-γ treated bone marrow derived macrophages was due IFN-γ driven induction of the host metabolite itaconate. Itaconate did not directly affect replication of LVS, but rather its impairment of complex II of the mitochondrial electron transport chain was attributed to restriction of LVS. The in vitro observation of itaconate-mediated control of LVS was confirmed in vivo as evident by mice deficient in their ability to produce itacontate to have reduced mean time to death and poorly control replication of LVS compared to wild type controls. In contrast, the highly virulent strain SchuS4 suppressed the ability of IFN-γ to trigger itaconate production and was resistant to IFN-γ mediated killing. These findings were underscored by the ability of SchuS4 to maintain mitochondrial function in the presence of IFN-γ. Direct addition of metabolites that impaired complex II, subverting the requirement for IFN-γ induced production of these compounds, readily controlled SchuS4 replication. Finally, treatment with FDA approved drugs known to impair mitochondrial function also controlled SchuS4 infection. Together these data describe a novel mechanism by which IFN-γ mediates control of bacterial replication and identifies new targets for development of novel therapeutics for intracellular pathogens.

Presenter: Bunis, Daniel

Transcriptional profiling with single-cell resolution reveals distinct fetal, newborn, and adult phenotypes of human naive T cells and their hematopoietic stem cell progenitors

Daniel Bunis, Rachel Lena Rutishauser, Ventura Mendoza, Chun Jimmie Ye, Trevor D. Burt
University of California, San Francisco

During development in utero, many human fetal immune cells are predisposed towards tolerogenic responses. Compared to adult human counterparts, human fetal naive CD4 T cells exhibit a higher propensity for differentiation into tolerogenic regulatory T cells (Tregs), and human fetal hematopoietic stem and progenitor cells (HSPCs) generate CD4 T cell progeny that are predisposed to Treg differentiation. After birth, naive T cells must transition toward a more mature state that supports protection against pathogens and cancer. At the time of birth, it is unknown whether adult-associated protective programs are fully expressed, either universally within most naive T cells, or heterogeneously within just a subset. Here, through single-cell transcriptional profiling of both naive T cells and HSPCs, from fetal, full-term newborn umbilical cord blood, and adult human sources, we demonstrate that the fetal to adult transition is incomplete across most cord blood naive T cells and their hematopoietic stem cell progenitors. Cells expressing a fully adult-like transcriptional signature are rare in cord blood, while most naive T cells and HSPCs exhibit a distinct, intermediate transcriptional state. In complementary bulk transcriptional profiling of naive CD4 T cells from the same samples, we also determined that the transcriptional state of naive CD4 T cells in cord blood includes intermediate expression levels of Treg signature genes. Thus, cord blood naive CD4 T cells likely retain a partial, fetal-like, Treg-predisposition. Our results may provide a novel mechanism that could explain why many vaccines, which were largely designed around elicitation of immunity in the protection-predisposed adult immune system, show reduced efficacy in neonates. Continued analyses of how expression of specific gene programs vary across ages will be carried out with the translational aim of informing specific ways that targeted, tolerogenic or protective, therapeutic responses might be elicited in both cord blood transplant and neonatal vaccination interventions.

Presenter: Byrne, Ashley

Long-read Single Cell Sequencing reveals RNA Isoform diversity across single B cells

Ashley Byrne, Roger Volden, Charles Cole, Theron Palmer, Anna Beaudin, Camilla Forsberg, Richard E Green, Christopher Vollmers

University of California, Santa Cruz

Due to plummeting costs, short-read sequencing technology continues to dominate the field for interrogating whole RNA landscapes. However, due to fragmentation of full-length molecules, heavy computational tools are needed to estimate the transcriptome landscape. Despite this, there still remain limitations in evaluating RNA isoforms in short-read data due to long distant events. We recently employed a long-read cDNA sequencing approach using Oxford Nanopore Technology (ONT) to evaluate single B cells. Additionally, we have improved the Oxford Nanopore base accuracy using a new method called R2C2 (Rolling Circle Amplification to Concatameric Consensus). By combining both approaches we have been able to reconstruct accurate isoform-level transcriptomes across single B cells using our analysis pipeline MandalorION. We have applied our methods to evaluate single B cells and have discovered that much of the RNA isoform diversity observed can be found across B cell specific receptors. This could potentially have serious implications for immunotherapy design specifically for targeting B cell lymphomas.

Presenter: Callaway, Perri

Characterization of NK receptor expression on a subset of gamma delta T cells from a malaria endemic region

Perri Callaway, Perri Callaway, Lila Farrington, Emma Lutz, Felistas Nankya, Kate Naluwu, Kenneth Musinguzi, Margaret Feeney

UCSF

Vγ9Vδ2 T cells (Vδ2s) are a subset of gamma delta T cells that recognize small non-peptidic molecules called phosphoantigens (pAgs) via their TCR. *P. falciparum*, the causative agent of malaria, produces pAgs in the plasmodial apicoplast. During in vivo *P. falciparum* infection, Vδ2s can expand to up to 50% of the circulating T cells in the peripheral blood. However, the frequency of Vδ2s has been shown to decrease after repeated malarial episodes and high exposure to P. falciparum. In contrast, expression of the FcγR, CD16, on Vδ2s increases. Previous lab data has indicated other natural killer receptors are also upregulated on Vδ2s from individuals with high P. falciparum exposure. Among these is the killer immunoglobulin-like receptor (KIR) family. We found that KIR expression varies between NK cells and Vδ2s from the same individual and KIRs are more highly expressed on CD16+ Vδ2s. Further, Vδ2s expressing KIR2DL4 were more likely to degranulate in response to *P. falciparum* antigens.

Presenter: Chao, Jaime L.

Impact of regulatory T cells on carcinogenesis of oral squamous cell carcinoma

Jaime L. Chao, Peter A. Savage
University of Chicago

Although some head-and-neck squamous cell carcinomas (HNSCCs) are associated with human papillomavirus (HPV) infection, a large proportion of HNSCCs are HPV-negative and associated with heavy smoking, suggesting that HPV-negative HNSCCs are induced by carcinogen exposure. In many human cancers, including HNSCC, regulatory T (Treg) cells are found at elevated densities and are thought to be a major barrier to the generation of robust anti-tumor T cell responses. However, recent studies indicate that Treg cells can serve diverse functions in non-lymphoid sites. To study the functional impact of Treg cells on the development and progression of HPV-negative HNSCC, we utilized an autochthonous mouse model of carcinogen-induced oral squamous cell carcinoma (SCC), in which mice are exposed long-term to the carcinogen 4-nitroquinoline-1-oxide (4-NQO) in the drinking water. Immunohistochemistry and flow cytometric analysis revealed elevated densities of CD3+ T cells and Foxp3+ Treg cells in pre-malignant dysplastic lesions and SCC lesions, suggesting that many lesions exhibit a T cell-inflamed phenotype. Single-cell TCR sequencing demonstrated the presence of recurrent Treg cell clones within carcinogen-induced lesions, suggesting clonal expansion of Treg cells in the tumor microenvironment. Surprisingly, systemic depletion of Treg cells at the later stages of carcinogen exposure did not induce tumor regression, but instead induced the opposite effect, driving increased incidence and burden of SCC. These findings suggest that Treg cells may play an unexpected role in restricting the progression of carcinogen-induced SCC in the oral cavity, prompting a re-examination of the common paradigm that Treg cells promote tumorigenesis by suppressing anti-tumor immunity.

Presenter: Chiu, Honyin

Effects of Elf4e Gene Dosage in Murine Primary B cells and Leukemia Cells

Honyin Chiu, Leandra V. Jackson, Sharmila Mallya, David A. Fruman
Benaroya Research Institute

The eukaryotic translation initiation factor 4E (eIF4E) protein binds to the 7-methylguanosine cap present in most mRNAs, recruits the scaffolding protein eIF4G and other proteins to form the translation initiation complex known as eIF4F. In cancer, eIF4F contributes to progression of the disease by preferentially translating mRNAs involved in sustained proliferative signaling, evasion of growth suppression, resistance to programmed cell death, replicative immortality, angiogenesis, invasion and metastasis. This makes cap-dependent translation an attractive target for cancer therapy, and efforts are underway to develop small molecule inhibitors of eIF4E, eIF4G and eIF4A for oncology. In lymphocytes, the 4E-binding protein (4E-BP)/eIF4E axis coordinates cell growth and proliferation during lymphocyte activation and our lab found that eIF4E activity is important for B cell differentiation and antibody class switching. This pathway is important in both normal B cell function and tumorigenesis; thus, we wanted to test the effects of reduced eIF4E protein levels in these parallel systems. Our findings show that reduced eIF4E protein slows tumorigenesis in a mouse model of pre-B cell leukemia, while normal mouse primary B cells can maintain B cell proliferation and antibody class switching. These findings establish that altering eIF4E dosage has significant yet selective biological consequences during tumor progression and supports studies showing that cancer cells are more sensitive than non-transformed cells to reductions in eIF4E protein.

Presenter: Cuevas, Victor D.

Investigating tissue-specific roles of IL-1 in behavioral and physiological metabolic adaptations during infection

Victor D. Cuevas, Janelle Ayres

Salk Institute for Biological Studies

Infections cause dramatic behavioral and physiological changes in the host that will affect host metabolism including an anorexic response, muscle and fat wasting. Infection also leads to modifications in the temperature set point of the organism to generate hyper- and hypothermic responses. Changes in thermoregulation are associated with changes in feeding behavior and changes in fat and muscle physiology. Cold exposure of mice increases feeding and metabolic rate when compared to mice housed at thermoneutrality. Moreover, cold-induced thermogenesis leads to depletion of energy stores likely to provide fuel to sustain the required temperature. The innate immune system is key not only in the defense against pathogens, but also in the regulation of metabolism, feeding and temperature. Intriguingly, the innate immune system is also central in the regulation of cold-induced thermogenesis. IL-1 signaling has been implicated in these physiological processes including the induction of anorexia, thermoregulation and mobilization of fat and muscle stores by acting via the nervous system. We have generated novel tissue specific transgenic mice that lack IL-1 signaling in the vagus nerve and hypothalamus as well as in adipose tissue to elucidate how the innate immune system orchestrates the interactions between all these different responses during infection and thermoregulation and how decisions are made to redirect the energy supply during infection.

Presenter: De Paula, Viviane

NMR solution dynamic in Interleukin-2 cytokine reveals allosteric coupling between JES6-1 antibody and CD25 receptor binding

Viviane De Paula, Kevin M. Jude, Jamie B. Spangler, K. Christopher Garcia, Nikolaos Sgourakis

University of California-Santa Cruz, UCSC

Interleukin-2 (IL-2) is a pleiotropic cytokine that regulates immune cell homeostasis and has been used to treat a range of disorders including cancer and autoimmune disease. IL-2 signals via interleukin-2 receptor-β (IL-2Rβ):IL-2Rγ heterodimers on cells expressing high (regulatory T cells, Treg) or low (effector cells) amounts of IL-2Rα (CD25). Previous studies have shown that interaction of mouse IL-2 with an anti-mouse IL-2 monoclonal antibody (JES6-1) preferentially enhance Treg populations through a unique mechanism whereby IL-2 is exchanged from the antibody to IL-2Rα. Using CPMG NMR relaxation dispersion experiments we characterized a dynamic conformational equilibrium of IL-2 with implications for the JES6-1 and CD25 binding. Additionally, using methyl-HMQC NMR we structurally characterized the IL-2/JES6-1 and IL-2/CD25 complexes to elucidate the distinct allosteric mechanisms through which these molecules modulate IL-2 function. Our results suggest that conformational exchange observed in the free IL-2 state involves a transient twisting or “breathing” of the α-helices, which may induce negative allosteric coupling between the JES6-1 antibody and IL-2Rα receptor binding sites, through the selection of distinct conformations from an ensemble of states sampled by the free form. These results provide a molecular basis for an allosteric mechanism in IL-2 signaling with potential implications for immunotherapy.

Presenter: Debenham, Luke

Developing a new assay to measure antibody affinity in response to vaccination among Common Variable Immune Deficiency patients

Luke Debenham, Michael Price (MSc), Dr Alex Richter and Prof. Kai Michael Toellner.
University of Birmingham

Background: Common Variable Immune Deficiencies (CVIDs) are a poorly categorized, heterogeneous group of conditions with unknown etiology, accounting for the majority of antibody deficiency cases. It is known that some patients produce normal titers of antibodies in response to vaccination, therefore, vaccination is given to prevent infection among CVID patients. Despite this, CVID patients remain susceptible to infection. Aims: We hypothesised that CVID patients with normal vaccine specific antibody titres would produce antibody of lower affinity than healthy controls. However, current methods of measuring of affinity towards complex antigens, such as chaotrope assays, are ineffective; and more accurate methods, such as surface plasmon resonance, are often prohibitively expensive. To address this, we are developing, optimizing and validating a novel measure of relative affinity by indirect competition enzyme-linked immunosorbent assay (icELISA). Methods/rationale: To measure affinity, known masses of monoclonal antibody specific to 4-Hydroxy-3-nitrophenylacetyl (NP) or tetanus toxoid (TT) are mixed with sera containing known masses of NP or TT specific antibody, subsequently, the monoclonal antibodies are labeled with alkaline phosphatase conjugated species. Sera with higher affinity antibodies will therefore have a greater ability to out-compete monoclonal antibody than lower affinity antibody. Results: Optimization and affinity measurements of sera using the icELISA concept have been achieved in NP immunized mice. We have also begun measuring TT specific antibody affinity from CVID patient sera, and we expect to make significant clinical observations soon. Future: We envisage that icELISAs will have wider applications monitoring the germinal centre response to complex antigens.

Presenter: Deets, Katie

Investigating a role for the NAIP/NLRC4 inflammasome in generation of an adaptive T cell response

Katie Deets, Russell Vance

UC Berkeley

The NAIP/NLRC4 inflammasome is a powerful tool of innate immunity that aids in the elimination of cytosolic bacteria. Several groups have looked into the role of inflammasome activation on adaptive T cell responses, but that work has largely been done in the context of bacterial infections, which make isolating the role of NAIP/NLRC4 difficult. The Vance lab has developed a novel in vivo genetic system where we can specifically induce expression of 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 cells under the control of a tamoxifen-inducible Cre recombinase. We will use this tool to determine if NAIP/NLRC4 activation is sufficient to generate an adaptive T cell response by addressing the following questions: (1) Are antigens released from pyroptotic cells taken up and displayed by antigen presenting cells? (2) What type of adaptive T cell response is generated? (3) Is this adaptive T cell response protective against challenge with flagellin-expressing pathogens?

Presenter: Delahaye, Jared

Alveolar and monocyte-derived macrophages differentially engage antibacterial programs during adaptive immunity to Mycobacterium tuberculosis.

Jared Delahaye, Courtney Plumlee, Christopher Plaisier, Sara Cohen, Eliza Peterson, Nitin Baliga, Kevin Urdahl

University of Washington

Cognate interactions between antigen-specific CD4 T cells and *Mycobacterium tuberculosis* (Mtb)-infected cells are critical for mucosal immunity to tuberculosis (TB). However, Mtb resides intracellularly within a variety of myeloid cell populations, and the relative impact of CD4 T cells on distinct infected cell types remains unknown. Here we report that intratracheally-transferred, Mtb-specific T cells are unable to reduce the bacterial burden in the first week after aerosol infection, when Mtb resides almost exclusively within alveolar macrophages (AMs). However, transferred T cells do mediate protection by day 14, at which point Mtb has disseminated to monocyte-derived macrophages (MDMs). Using microscopy in combination with a fluorescent reporter strain of Mtb, we observe that AM harbor more bacteria than MDM on a per cell basis even after T cells traffic to the lung. RNA-Seq analysis of these two macrophage populations reveals early and sustained enrichment for proinflammatory pathways (i.e. NFkB signaling) in Mtb-infected MDMs compared to AMs. In parallel, we analyzed the bacterial transcriptome and found that Mtb from AM engage distinct response programs compared to Mtb from MDM. Together, our results indicate that AMs may serve as a privileged niche for Mtb, despite the presence of Mtb-specific T cells. As many current TB vaccine candidates seek to induce lung-resident T cells that can recognize and control Mtb early after aerosol infection, the relative resistance of Mtb-infected AMs to T cell-mediated immunity could represent a previously unappreciated barrier to this strategy.

Presenter: DeRogatis, Andrea

Modification of a Novel Immune Assay for use with Japanese Quail (Coturnix japonica)

Andrea DeRogatis, Kirk C. Klasing
University of California, Davis

Knowledge about an organism's immune system is important to a variety of biological sciences, not just the specific field of immunology. Although there are a variety of techniques available to study the avian immune system, there are also currently numerous limitations. Thus, there is a need for research that builds on existing methods and develops new techniques that can be used to study all components of the avian immune system in both laboratory and field environments. The main objective of this study was to determine if a novel immunological assay, designed for chicken samples, could be modified using quantitative polymerase chain reaction (qPCR; aka real-time PCR or RT-PCR) analysis to make it applicable for samples from Japanese quail (Coturnix japonica). Secondary to the main aim was to determine whether lipopolysaccharide (LPS) dose, time of feather removal, or injection site significantly influenced the innate immune response as reflected by measurable changes in interleukin-1-beta expression (IL1β). Two experiments were performed to address these aims. In the first experiment, blood feathers on both the left and right wings of Japanese quail were injected with either 1X concentration endotoxin free phosphate buffered saline (PBS), a medium (0.1 μg) or high dose (1 μg) of LPS. The same experimental design was used for the second experiment, however only feathers from the right wing were injected. Using quantitative RT-PCR, we were able to successfully track changes in IL1β expression level in the feather tissue (pulp) in response to LPS injection. In both experiments, there were significant effects of time ($P < 0.001$), treatment ($P < 0.001$) on IL1β expression. These results demonstrate that qPCR can be used to successfully measure an important modulator of an innate immune response using tissue from the growing feathers of Japanese quail. This technique will provide researchers with a valuable tool that can be used to help address avian-based research questions important to numerous fields ranging from ecology to immunology.

Presenter: Dolina, Joseph S.

Endogenous release of mammalian DNA during microbial infection triggers TLR9-mediated immunosuppression of cytotoxic T lymphocytes

Joseph S. Dolina, Joey Lee, Ryan Q. Griswold, and Stephen P. Schoenberger

La Jolla Institute for Immunology

It has long been appreciated, but not understood, that the CD8+ cytotoxic T lymphocyte (CTL) dependence on conventional CD4+ T cell “help” is conditionally needed for some immunogens but not others. The quality of early inflammatory events downstream of innate cues that evolved to sense “danger” is not only thought to bypass the need for “help” thus having a direct effect on CTLs, but can also promote and suppress CD4+/CD8+ T cell immunity in a context-dependent manner. Here we show that the function and magnitude of the CD8+ T cell response to Listeria monocytogenes (Lm) infection is controlled by a functionally heterogeneous CD4+ T cell population re-shaped by inflammation associated with bacteria infectious dose. Low infectious dose induces an antigen-specific CTL response profoundly dependent on “help”, whereas high infectious dose results in a CTL response significantly inhibited by CD4+CD25+FoxP3+ regulatory T cells (Treg) converted from conventional FoxP3- precursors. Suppression of CTLs by and conversion to functional Treg is dependent on both early TLR9- and IL-12-mediated inflammation relayed through the canonical CD8a+ dendritic cell. The extent of TLR9-mediated suppression is directly related to the quantity of self-DNA release into peripheral circulation as a result of dose-related tissue damage early during infection. Our data hereby reveal that the CTL response to the same pathogen is determined by distinct roles of conventional CD4+ T cells differentiated into helpers or suppressors by the extent of TLR9 ligation to self-DNA and associated inflammation.

Presenter: Domeier, Phillip

Exclusive expression of an exon 2-deficient Foxp3 isoform promotes IgE-driven cutaneous autoimmunity

Phillip Domeier, Jianguang Du, Sabine Spath, Baohua Zhou and Steven Ziegler

Benaroya Research Institute

In humans, the FOXP3 gene encodes multiple protein products from alternative splicing of the mRNA transcript, but mice only encode the full-length isoform. Regulatory T cells (Tregs) that express the exon 2-deficient isoform (FoxP3.ΔExon2) elicit impaired Treg function. Furthermore, in humans, the development of autoimmunity correlates with elevated expression of the FoxP3.ΔExon2 isoform by an undefined mechanism. To determine how the FoxP3.ΔExon2 isoform contributes to autoimmunity in vivo, we developed mice that express an exon 2-deficient isoform of the FoxP3 gene (called B6.FoxP3.ΔExon2). B6.FoxP3.ΔExon2 mice develop mild systemic autoimmune disease, characterized by splenomegaly, cutaneous lesion formation and mild kidney nephritis. Furthermore, these mice exhibit defective T follicular regulatory (Tfr) cell activity, resulting in the development of increased germinal center frequency and elevated serum IgE titers in the absence of overt infection or immunization. As compared to wild-type (C57BL/6, B6) littermates, B6.FoxP3.ΔExon2 mice have elevated Keratin 14-reactive IgE, increased deposition of IgE in the keratinocyte layer of the skin and greater numbers of IgE-producing cells in skin-draining lymph nodes. Collectively, we show that exon 2 region of the mouse Foxp3 gene is crucial for regulation of germinal center-derived IgE autoantibody formation and IgE-driven cutaneous disease.

Presenter: Dulson, Sarah

STAT4 Directs Protective Innate Lymphoid Cell Responses to Gastrointestinal Infection

Sarah Dulson, Emily Watkins, and Laurie E. Harrington, Ph.D.
University of Alabama at Birmingham

Foodborne infection with Listeria monocytogenes (Lm) carries a mortality rate of 20-30% in high-risk individuals and leads to serious complications such as meningitis and miscarriage. Therefore, studies evaluating gastrointestinal responses to Lm are needed to better enhance therapeutic interventions. Our data show that adaptive immune responses are dispensable in the first 5 days of infection since bacterial burden is similar between Rag1-deficient and wildtype mice. Innate Lymphoid Cells (ILCs) are tissue-resident lymphocytes that are enriched at barrier surfaces, and we hypothesize that intestinal ILCs are central mediators of the early response to Lm infection. Using cytokine reporter mice, we demonstrate a robust and early IFNγ response to Lm by Group 1 ILCs (ILC1s) in the large intestine lamina propria. In addition, the systemic IFNγ response is significantly reduced in the absence of ILCs. Consistent with this, mice devoid of ILCs mice suffer higher mortality and increased bacterial dissemination compared with ILC-sufficient littermates. Mechanistically, ILC1s lacking the transcription factor STAT4 are unable to produce IFNγ, and STAT4-deficient mice readily succumb to bacterial dissemination and mortality following Lm infection. Interestingly, STAT4-deficient animals with intact ILC populations were no longer protected against infection compared with ILC-depleted littermates, indicating that STAT4 signaling is critical for ILC-mediated protection. Furthermore, inhibition of STAT4 activity immediately prior to cytokine stimulation demonstrates that STAT4 directly promotes IFNγ production in ILC1s. Together, these data illustrate a critical role for ILCs in the early responses to gastrointestinal infection with Lm and identify STAT4 as a central modulator of ILC-mediated protection.

Presenter: Evans, Katrina

Single- Cell Analysis of the Brain Microenvironment in Breast Cancer Metastasis

Katrina Evans, Kerrigan Blake, Quy Nguyen, Hamad Alshetaiwi, Kai Kessenbrock, Devon A Lawson

University of California Irvine

Breast cancer brain metastasis affects some 10-20% of patients, and there are very few effective treatments. The native immune effectors in the brain are macrophage-like cells called microglia, and astrocytes. These cell types represent close to 50% of cells in the brain and function to survey the blood brain barrier (BBB) for disruptions, protect the brain from invading pathogens and respond to injury and inflammation. Activated infiltrating microglia and astrocytes are a major component of metastatic breast tumors in the brain, but it is not known whether these cells are involved in the promotion or rejection of breast cancer cells. To address this gap in knowledge we isolated microglia, macrophages and astrocytes from metastatic and control mouse brains and generated single cell RNA libraries to interrogate their gene expression. Using the Seurat analysis pipeline we have identified metastasis associated microglia gene signatures are similar to microglia in other neuroinflammatory and neurodegenerative diseases.

Presenter: Fois, Adrien

Modulation Mitochondrial Respiration Alters Immune Homeostasis at Steady State

Adrien Fois, Claudine Beauchamp, Genevieve Chabot-Roy, Guy Charron, Yan Burelle, Christine Des Rosiers, John D. Rioux, Sylvie Lesage

Hopital Maisonneuve Rosemont

Leigh syndrome French Canadian type (LSFC) is an inheritable disease characterized by the occurrence of acute crises of fulminant metabolic acidosis, which considerably increase disease severity and mortality. This syndrome is caused by a mutation in the LRPPRC gene. LRPPRC regulates mRNA stability of many mitochondrial proteins necessary for the assembly of the respiratory chain. As such, mutations in LRPPRC modulate mitochondrial respiration, known to polarize immune responses. Interestingly, metabolic crises in LFSC patients often follow infections. These results highlight the possible contribution of the immune system in the onset of metabolic acidosis crises. To investigate the role of LRPPRC in immune function, we generated various mouse models. Due to the embryonic lethal phenotype of LRPPRC-/- mice, we generated LRPPRCfl/fl x Mx1-Cre mice. Deletion of LRPPRC in adult mice results in a depletion of many immune cells, including NK cells and plasmacytoid dendritic cells. In an attempt to better understand the impact of the LSFC causal mutation, we also generated LRPPRC-354V-KI mice. Mice homozygous for the A354V mutation die in utero. However, we were able to analyze LRPPRC-354V-KI+/- and observed an increase in T cells when compared to WT mice. Altogether, our preliminary data suggest that a deficiency in LRPPRC, which modulates mitochondrial respiration, influences immune homeostasis. We are currently investigating its role in ongoing immune responses. It remains to be seen whether these observations will be generalizable to other, less severe, metabolic syndromes.

Presenter: Glassman, Caleb

An IL-2 receptor partial agonist expands FoxP3+ regulatory T cells in vivo

Caleb Glassman, Leon Su, Sonia Majri, K. Christopher Garcia
Stanford University

IL-2 is critical for the development, survival and effector function of multiple lymphoid cell populations. Using mutations previously identified by our group that impair interactions with the common gamma chain, we generated a series of IL-2 receptor partial agonists and assessed their activity in vivo. One mutein, REH, showed specific activity on FoxP3+ regulatory T cells with reduced activity on CD8s. This mutein mirrored Treg specific difference in IL-2 signaling with increased dependence on the high-affinity IL-2 receptor, CD25, and heightened sensitivity to negative regulation by suppressor of cytokine signaling 1 (SOCS1). Consistent with its effect on Tregs, REH administration delayed disease onset and decreased disease severity in the context of experimental autoimmune encephalomyelitis. Together, these results demonstrate that intrinsic differences in IL-2 sensitivity can be exploited to generate variants with cell-type specific activity.

Presenter: Goldberg, Emily

Ketogenic diet activates protective [g][d] T cell responses against influenza virus infection

Emily Goldberg, Ryan D. Molony, Sviatoslav Sidorov, Eriko Kudo, Vishwa Deep Dixit, and Akiko Iwasaki

Yale University

Influenza A virus (IAV) infection-associated morbidity and mortality are a key global healthcare concern, necessitating the identification of novel therapies capable of reducing the severity of IAV infections. In this study, we show that the consumption of a low-carbohydrate, high-fat ketogenic diet (KD) protects mice from lethal IAV infection and disease. KD feeding resulted in an expansion of γδ T cells in the lung that improved barrier functions, thereby enhancing anti-viral resistance. Expansion of these protective γδ T cells required metabolic adaptation to a ketogenic diet, as neither feeding mice a high-fat high-carbohydrate diet nor providing chemical ketone body substrate that bypasses hepatic ketogenesis protected against infection. Therefore, KD mediated immune-metabolic integration represents a viable avenue towards preventing or alleviating influenza disease.

Presenter: Goretsky, Yitzhar

High-Affinity Auto-reactive Double Negative Thymocytes Can Escape Deletion and Migrate to Peripheral Lymphoid Organs

Yitzhar Goretsky, Nicholas Fong, Martha C. Zuñiga, Shahar Dubiner, Alicia Freedman, Bryan Kim, Alisan Sas, Stefan Abreo, Jordan Schneider, and Tyler-Marie Deveau

University of California, Santa Cruz

Negative selection of developing thymocytes is the major mechanism for developing immune tolerance to self. We have studied the development and function of T cells expressing the BM3.3 TCR of transgenic CBA (H-2k haplotype) mice. The BM3.3 TCR has high affinity for the alloantigen, H-2Kb. H-2Kb can induce signaling in naive BM3.3 T cells in a CD8-independent manner. Moreover, deletion of BM3.3 thymocytes occurs in the absence of CD8, indicating that the interaction of the BM3.3 TCR with H-2Kb alone is sufficient for negative selection. Mellor's group previously showed that negative selection of BM3.3 T cells occurs in a double transgenic model in which H-2Kb expression is driven by the guinea pig alpha-lactalbumin promoter (KALxBM3 mice). We have examined in greater detail the development of thymocytes in KALxBM3 mice. We found that there are few CD8+ thymocytes, but the CD4+ thymocytes do not occur in greater numbers in KALxBM3 mice than in parental strains. Surprisingly, the cellularity of KALxBM3 thymii is ~70% of that of parental KAL or BM3 mice. This led us to examine the CD4+CD8+ (DP) and CD4-CD8- (DN) thymocytes. We observed a four-fold greater percentage of DN thymocytes in KALxBM3 mice than in BM3 mice. The high affinity of BM3 TCR for its H-2Kb ligand and its ability to signal in the absence of CD8 suggested to us the possibility that some of the DN thymocytes in KALxBM3 had engaged H-2Kb. An increase in CD5 expression is commonly regarded as evidence of the interaction of a thymocyte with its cognate antigen. We used multi-color flow cytometry to examine the CD5 phenotype of all thymocyte populations in young adult mice. We found that in addition to normal immature CD5lo DN thymocytes KALxBM3 thymii contain DN cells having elevated levels of CD5. These â€œantigen-experiencedâ€← DN thymocytes can be distinguished from immature DN thymocytes in that they are also Ti98hi (i.e., express high levels of the BM3 TCR) and CD62Lint. These CD5hiTi98hiCD62Lint antigen-experienced DNs make up ~23% of the DN thymocyte population in KALxBM3 mice. To determine if these thymocytes contribute to mature T cell populations we examined lymph node T cells from KALxBM3 mice. 4.7% of the CD3+ lymph node cells express neither CD4 nor CD8. These DN T cells express high levels of the Ti98-reactive BM3 TCR. Interestingly, they also express high levels of CD62L and CD44, markers of the memory phenotype. Taken together these results show that DN thymocytes expressing a high-affinity TCR can proceed through thymic development, and escape deletion. They can mature and migrate to peripheral lymphoid organs where they may influence immune responses.

Presenter: Hatton, Olivia

Regulation of Host microRNA Expression by Natural Variants of Epstein-Barr Virus (EBV) Latent Membrane Protein 1 (LMP1)

Olivia Hatton, Aleishia Harris-Arnold, Abbie Hui, Eden Maloney, Sheri Krams, Olivia Martinez

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Malignancies attributable to EBV, such as post-transplant lymphoproliferative disorder (PTLD), represent 1.8% of global cancer deaths. PTLD occurs in 1-20% of transplant recipients and results in significant morbidity and mortality. The mechanisms the virus utilizes towards the development and progression of EBV+ B cell lymphomas like PTLD are poorly characterized. We previously demonstrated that EBV infection modulates expression of host B cell microRNAs (miRs), small non-coding RNAs that regulate gene expression. To determine how the EBV oncogene latent membrane protein 1 (LMP1) regulates expression of host cell miRs, we used EBV- B lymphoma lines that express inducible, chimeric LMP1 molecules derived from the B95.8 lab strain of EBV and PTLD tumor variants previously defined to contain gain of function mutations at position 212 and 366 of LMP1. miR-155 and miR-886-5p were significantly upregulated in B cells by EBV infection and both B95.8 and tumor variant LMP1. However, while miR-193b was upregulated by EBV infection and B95.8 LMP1, it was not modulated by PTLD tumor variant LMP1. To evaluate the requirement for specific signal transduction pathways, we utilized small molecule inhibitors (SMI) to target NFκB, p38, ERK, mTOR or PI3K. NFκB and p38 signaling were required for miR-155, but not miR-193b or miR-886-5p upregulation. Notably, miR-155 upregulation was also significantly reduced by a PI3Kα,δ inhibitor and PI3Kα-specific inhibitor. Compared to EBV- B cell lymphoma cells and associated exosomes, miR-155 and miR-193b, but not miR-886-5p, is significantly elevated in EBV+ B cell lymphomas and their associated exosomes. Moreover, EBV+, but not EBV-, B cell lymphomas, do not express the miR-155 target FOXO3a. Our data suggests that host miR expression by LMP1 is altered in PTLD and that unique signaling pathways downstream of LMP1 are required to regulate the expression of each host miR. Finally, this is the first demonstration that LMP1 regulates miR-155 expression by the PI3K pathway, specifically the PI3Kα isoform. Thus, modulation of miRNA by SMI is a potential therapeutic approach for EBV+ PTLD and exosomal miR-155 and miR-193b may serve as biomarkers for EBV+ B cell lymphomas like PTLD.

Presenter: Hobbs, Samuel

Targeted expansion of tissue-resident CD8+ T cells to boost cellular immunity in the skin

Samuel Hobbs, Jeffrey Nolz
Oregon Health & Science University

Effective cellular immunity against intracellular pathogens requires direct recognition of peptide-MHC by CD4+ and CD8+ T cells. Therefore, protective memory T cells must either be already positioned at the site of pathogen entry or be able to rapidly localize to inflamed tissue microenvironments following re-infection. Traditionally, the goal of vaccination strategies targeting the durable formation of cellular immunity has focused on generating large populations of circulating antigen-specific memory T cells using booster immunizations and/or strong adjuvants. However, in many human vaccination trials, the numbers of circulating memory T cells do not correlate with protection. This lack of protection by circulating memory T cells has generated a strong interest in developing vaccines that seed tissue-resident memory (TRM) T cells at sites of pathogen entry. Although their protective capacity is well established, whether pre-existing TRM populations can be specifically boosted to increase tissue-specific protective immunity is largely unknown. Here, we demonstrate that repeated activation of rare, endogenous TRM CD8+ T cells using only topical application of antigenic peptide caused delayed-type hypersensitivity and increased the number of antigen-specific TRM CD8+ T cells specifically in the challenged skin by ~15-fold. Expanded TRM CD8+ T cells in the skin were derived from memory T cells recruited out of the circulation that became CD69+ tissue-residents following local antigen encounter. Notably, recruited circulating memory CD8+ T cells of a different antigen-specificity could be coerced to become tissue-resident using a dual peptide challenge strategy. This “recruit and capture” technique significantly increased anti-viral protection in the skin, suggesting this procedure could be used to rapidly boost tissue-specific cellular immunity. Overall, our study demonstrates that established TRM T cells can be amplified in a tissue-specific manner to rapidly enhance protective cellular immunity without relying on booster immunizations or adjuvants, but also suggests a mechanism of how pathogenic T cells specific for commensal or environmental antigens could continually accumulate in the skin following repeated exposures.

Presenter: Hoyer, Katrina

CD8 T follicular cells amplify germinal center B cell reactions enhancing autoimmune disease

Katrina Hoyer, Kristen M. Valentine, Genevieve N. Mullins, Travis J. Lawrence

University of California Merced and Oak Ridge National Laboratory

We and others have identified a novel CXCR5+PD-1+CD8 effector T cell (CD8 follicular T cells; CD8 Tfc) that migrates into the B cell follicle, expresses B cell stimulatory proteins, and stimulates B cells to differentiate and antibody isotype switch, but also expresses cytolytic enzymes, expands in response to cancer, and targets virus-infected cells for destruction. The role of CD8 Tfc cells has only just begun to be explored, and as such the signaling cascades and cytokine signals that drive their function have not been defined. During autoimmunity CD8 Tfc may promote germinal center-derived reactions, or target cells for destruction. Our objective is to ascertain the functional role of CD8 Tfc cells in germinal center and extrafollicular reactions during autoimmune disease. We hypothesize that CD8 Tfc cells that arise under autoimmune conditions promote B cell germinal center function via overlapping and unique mechanisms to CD4 Tfh cells. We will address CD8 Tfc cell function within the follicle, and the signals, cytokines and interactions underlying these activities on B cells. We will assess the contact-dependent and -independent signaling pathways induced in B cells by interactions with CD8 Tfc cells, and the subsequent impact on B cell function. CD8 Tfc cells may control germinal center reactions through the induction of activation-induced cytidine deaminase, class-switch recombination, and plasma cell differentiation. Because CD8 Tfc cells function to promote B cell germinal center responses, our results impact the study of chronic inflammatory diseases associated with extensive antibody production such as lupus, allergy and arthritis. Funding: NHLBI grant R15HL146779-01

Presenter: Huang, Hsin-I

Paradoxical pro-tumor functions of dendritic cells on colorectal cancer

Hsin-I Huang, Gianna Hammer

Department of Immunology, Duke University

The immune system's ability to eradicate cancer requires coordination between innate and adaptive immune cells. No other cell type bridges innate and adaptive immunity better than the dendritic cell (DC). For this reason, DCs, specifically the Batf3-dependent DC subset, are thought to be key to anti-tumor immunity. Although the anti-tumor functions of Batf3-dependent DCs have been well described in skin cancer, whether these DCs have similar anti-tumor functions in other cancers is unknown. Here we investigated the roles of Batf3-dependent DCs in colon cancer and paradoxically found that these DCs actually promote colon tumorigenesis. Thus, in a spontaneous colorectal cancer mouse model, loss of Batf3-dependent DCs resulted in decreased colon tumor number. Although Batf3-dependent DCs are best known for expanding IFNγ-producing T cells, in colon tumors, we surprisingly found that Batf3-dependent DCs were required to expand tumor-infiltrating γδT cells producing IL-17. IL-17 is known to promote colon tumorigenesis and our findings pinpoint IL-17-producing γδT cells as a key source of IL-17 for tumor growth, and surprisingly describe a requirement for Batf3-dependent DCs to expand an IL-17-producing T cell population. In contrast, we found that Batf3-dependent DCs were not required for expanding tumor-infiltrating IFNγ-producing T cells suggesting that anti-tumor cytokine IFNγ was not compromised after losing Batf3-dependent DCs. Furthermore, we identified Batf3-dependent DCs were required for γδT cells survival in colon tumor, as a potential mechanism that Batf3-depednent DCs regulated γδT cells. In addition to this function, we identified PD-L1 expression as a second mode of action by which Batf3-dependent DCs promote colon cancer. While we found that all DC subsets enter colon tumors, tumor-infiltrating Batf3-dependent DCs uniquely upregulate high levels of PD-L1. PD-L1 upregulation and high PD-L1 expression on Batf3-dependent DCs was specific within the tumor microenvironment. Importantly, PD-L1 was not expressed on colon tumor cells themselves, suggesting that PD-L1 expression on Batf3-dependent DCs may be dominant for PD-L1-mediated immunosuppression in colorectal cancer. Collectively, these paradoxical pro-tumorigenic functions and phenotypes of Batf3-dependent DCs suggest that these DCs are not universally anti-tumor and that targeted therapies that modulate Batf3-dependent DCs could benefit colorectal cancer patients.

Presenter: Huang, Jessica

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

Jessica Huang, Karan Kohli, Joseph Leal, 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 Type-1 inflammatory adjuvants to study the responses of different innate cell populations in the draining LNs. We found that within hours of immunization with distinct TLR agonists, monocytes rapidly migrated to the draining LNs in large numbers and differentiated into monocyte-derived DCs (MoDCs). These MoDCs further infiltrated the deep T cell zone where they physically interacted with T cells undergoing activation by cDCs. While MoDCs did not capture large quantities of antigen, they did constitute a major source of IL-12 production in the T cell zone, suggesting their likely role in T cell differentiation. Indeed, early-effector T-bet high T cells were preferentially enriched in regions heavily infiltrated by MoDCs, suggesting localized T cell polarization. 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 effector CD4+ and CD8+ T cells 4 days post-immunization. Together, these data suggest that during the generation of adaptive immune responses, monocytes in draining LNs create a localized spatial niche and cooperate with cDCs to promote the production of optimally differentiated effector T cells.

Presenter: Huang, Zhiyu

Developing PK/PD models to dissect MOA of IRAK4 signaling and discover novel IRAK4 SMIs

Zhiyu Huang, George Francis, Kevin DeMent, Le An, Ivan Peng, Swathi Sujatha-Bhaskar, Edna Choo, Yingqing Ran, Callie Bryan, James Kiefer, Ali Zarrin, Claire Emson, Brent McKenzie, Hans D. Brightbill

Genentech

Purpose: The IRAK4 kinase plays a critical role in initiating innate immune responses against foreign pathogens. It is a key signaling component downstream of most Toll-like receptors (TLR) and IL-1R, and when activated can elicit production of inflammatory cytokines, such as IFNα, IL-6, and TNFα. Blocking IRAK4 function could be of therapeutic benefit for autoimmune and inflammatory diseases, including SLE and asthma. Here we established several disease-relevant - IRAK4 dependent in vivo PK/PD models to facilitate development of IRAK4 small molecule inhibitors. Results: We successfully developed PK/PD models to test the role of IRAK4 kinase in signaling pathways of the innate immune response. These include, TLR7/8 agonist (R848) induced IFNα, TLR9 agonist (CpG) induced IL-6/TNFα, TLR9 agonist (CpG) with liposomal transfection reagent DOTAP induced IFNα, and IL-1-induced IL-6 production. IRAK4 kinase dead (KD) mice were used to validate the IRAK4 dependence of each of these models. In addition, transfer of IRAK4 KD bone marrow to wild type C57BL/6 mice failed to respond to R848 induced INFα in the chimera. Similarly, Antibody depletion of pDCs abolished R848 and CpG/DOTAP induced IFNa response, but left CpG induced IL-6/TNFa response intact, implicating pDCs as the cellular source of IRAK4 dependent INFα production in response to TLR stimuli. Finally, consistent with IRAK4 KD mice, IRAK4 tool compounds were able to inhibit multiple IRAK4 dependent PK/PD responses in a titratable fashion, which paved the way for screening of potent and selective IRAK4 compounds. Conclusion: We developed consistent and robust in vivo PK/PD models of IRAK4 dependent signaling downstream of innate immune cell receptors (TLR, IL1R). These in vivo models gave us greater understanding of IRAK4 biology and provided a robust means for screening IRAK4 small molecule inhibitors in vivo as potential therapeutics to treat diseases, such as Systemic Lupus Erythematosus and Asthma.

Presenter: Huggins, Matthew A.

Natural microbial exposure shapes the memory CD8 T cell pool and immune responsiveness to new challenges

Matthew A. Huggins, Mark Pierson, Sara E. Hamilton
University of Minnesota

Over time CD8 T cells encounter multiple infections independently from recognizing cognate antigen through their T cell receptor. Inflammatory events caused by microbial exposures can be quite numerous and may impact future immune responses to vaccines or resistance to infection. Although many studies have investigated how repeated antigen stimulation causes T cell exhaustion, repeated bystander inflammation has not been well characterized. In this study, we compare identical CD8 T cell populations in specific pathogen free (SPF) mice to those in “dirty” laboratory mice co-housed with microbially diverse pet store mice. Our published data indicate that dirty mice acquire a diverse set of microbes upon cohousing with pet store mice and exhibit increased pro-inflammatory gene signatures in the blood. In ongoing work, we demonstrate that cohoused mice have increases in baseline levels of numerous inflammatory cytokines and chemokines. These differences correspond with widespread changes in the cellular composition of immune cells. Specifically, cohousing skews the memory T cell population towards long-lived effector cells (LLEC). This subset is characterized by expression of terminal effector differentiation markers (KLRG1), cytotoxic molecules (granzyme B), and unique chemokine receptor expression (CX3CR1). Importantly, memory CD8 T cells isolated from dirty mice provide significantly improved pathogen clearance after intentional infection with Listeria monocytogenes (LM). Cohoused T cells provided enhanced protection to both antigen specific and antigen-independent challenges. Ongoing studies are focused on defining the extrinsic inflammatory signals that contribute to altered CD8 T cell functionality as well as the T cell intrinsic mechanisms of enhanced pathogen protection. A more complete understanding of how previous antigen exposure shapes the immune response could help optimize the vaccination and treatment of humans with varying degrees of microbial burden.

Presenter: Isho, Baweleta

Role of maternal mucosal immunity on progeny susceptibility to type 1 diabetes

Baweleta Isho, Leili Marandi and Philippe Poussier
University of Toronto and Sunnybrook Research Institute

Type 1 Diabetes (T1D) is a disorder of glucose homeostasis resulting from the destruction of insulin-producing pancreatic β-cells by T lymphocytes. Multiple genetic and environmental risk factors contribute to disease susceptibility. While environmental determinants remain elusive in humans, studies in the spontaneously diabetic NOD mouse have demonstrated that the differential susceptibility of males and females to disease is associated with the composition of the intestinal microbiome and its impact on systemic testosterone levels. Here, using the progeny of crosses between NOD and immunocompromised NOD.*scid* mice, we tested the hypothesis that maternal mucosal immunity plays a role in the pathogenesis of diabetes through its impact on microbiome composition. We observed that in the prediabetic stage, the number of animals with anti-insulin autoantibodies was lower in the progeny of NOD.*scid* mothers than in that of NOD mothers (13.3%, n = 15 vs 58.8%, n = 17, p = 0.008). Later, female mice born to NOD.*scid* mothers had a lower T1D incidence when compared to those born to NOD mothers (61.1%, n = 20 vs 95.0%, n = 20, $p<0.05$) while the maternal immune status did not influence disease incidence in male progeny born to NOD.*scid* or NOD mothers (43.4%, n = 25 vs 61.5%, n = 13, $p>0.05$). Strikingly, disease protection conferred by NOD.*scid* mothers was associated with changes in sex hormone levels in the plasma. Specifically, testosterone levels in male progeny of NOD.*scid* mothers were significantly lower than in males born to NOD mothers (1.0±1.5 ng/ml, n = 8 vs 10.7±11.3 ng/ml, n = 8, $p<0.05$). Similarly, plasma levels of the active estrogen, estradiol, were lower in female progeny of NOD.*scid* mothers than in that of NOD mothers (30.2±21.7 pg/ml vs 113.1±33.5 pg/ml, $p<0.05$). Ongoing studies are investigating the contribution of the intestinal microbiome and maternal secretory IgA responses to these hormonal changes and the modulation of islet inflammation.

Presenter: Jamieson, Amanda

Immune triage: Prioritization of the innate immune response when faced with multiple simultaneous insults.

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The innate immune system is responsible for a number of essential functions in the human body including tumour immunosurveillance, pathogen clearance, development, and wound healing. Many of these processes must happen simultaneously. We are interested in how the innate immune response prioritizes what it responds to when faced with multiple simultaneous insults. Our previous research has demonstrated how infection with influenza A virus impacts a secondary pulmonary infection with bacteria. We have expanded on this concept to show how viral or bacterial pulmonary infection alters the ability to heal wounds in a distal site. Retrospective analysis of clinical data showed that patients with pneumonia are at risk for poor wound healing. Using animal models of cutaneous wounds, we have demonstrated that lung infections suppress the infiltrate of leukocytes into the wound. As these cells are essential for the early stages of tissue repair there is a delay in the rate of wound healing. The innate immune response in the lung is not suppressed in the dual insult model, and pulmonary pathogens are cleared equivalently in wounded and unwounded mice. This demonstrates that lung infections are prioritized over cutaneous wounds, likely because maintaining a functioning lung is essential for survival. We are able to restore the rate of wound healing to that of uninfected mice by treating the wounds with chemokines that increase the amount of infiltrating leukocytes. Importantly, this study establishes new potential treatment possibilities for patients that are faced with complicated comorbidities. These data demonstrate a new biological premise when the innate immune response is responding to both an infectious and non-infectious insult. The concept of immune triage will be broadly applicable to many diseases that involve the innate immune response.

Presenter: Jeftic, Ilija

Age-related changes in organization and function of lymph node microenvironment

Ilija Jeftic, Nico A Contreras, Richa Jain, Heather L Thompson and Janko Nikolich-Å½ugich

Department of Immunobiology and the University of Arizona Center on Aging, University of Arizona College of Medicine-Tucson, Tucson, Arizona, USA

Elderly patients are more vulnerable to infectious diseases and show diminished responses to vaccination compared to adult counterparts. While age-related changes in primary lymphoid organs are well described, the morphological and functional changes affecting secondary lymphoid organs have been less well characterized. Lymph node (LN) structure, including its stromal cells, is crucial to its function, that consists of lymphocyte maintenance, as well as of directing antigen, antigen-presenting cells, and antigen-specific lymphocytes into intimate communication necessary to mount immune responses. Experimental evidence indicates that LNs undergo alterations during aging, including drastic reduction in both lymphoid and stromal LN cell numbers and organization. Additionally, we show an age-dependent replacement of areas populated with immune cells by connective tissue and an increased degree of fibrosis in LNs compared to adult counterparts. Aged LNs exhibited higher expression of fibrosis-related cytokines such as TGF-b. Treatment of aged animals with trophic factors reversed age-related lymph node defects and fibrosis, with an expansion of both stromal cells and naive T cells. Collectively, these results demonstrate an age-related increase in LN fibrosis, implicate profibrogenic pathways, and begin to establish targeted strategies to improve immune homeostasis, immune responses and vaccine efficacy in the elderly.

Presenter: Jo, Yeara

Genomic Analysis Of Bone Marrow Progenitors During Viral Infection Reveals Novel Dendritic Cell Regulators

Yeara Jo, Kai Zhang, Wei Wang, Elina Zuniga

University of California, San Diego

Dendritic cells (DC) play a central role in immune responses and can be broadly subdivided into conventional (c) as well as plasmacytoid (p) DCs. Notably, we and others have described several adaptations of DCs and their progenitors during acute and chronic infections, including impaired DC development, maturation and altered cytokine production. To understand the mechanisms underlying such adaptations we determined the transcriptional and chromatin landscapes of bone marrow (BM) DC progenitors from lymphocytic choriomeningitis virus (LCMV) infected mice, via RNA-Seq and ATAC-Seq, respectively. Initial analysis indicated that infection induced multiple alterations in gene signatures, including type-I-interferon signaling and metabolic pathways, as well as changes in chromatin accessibility, which were more enriched within intergenic regions. We next used the Taiji algorithm, which integrates chromatin accessibility and gene expression to assess the global importance of transcription factors (TFs) at the systems level. This analysis identified 11 known DC regulators and 24 TFs with no previous connection to DC biology. Follow-up knock-down experiments revealed that Glucocorticoid Modulatory Element Binding Protein 1 (Gmeb1), which was predicted to exhibit increased activity in progenitors from LCMV-infected mice, suppressed DC development and maturation. On the other hand, Zinc Finger Protein 524 (Zfp524), whose activity was predicted to be reduced in progenitors from LCMV-infected mice, promoted pDC cytokine production while inhibiting the same function in cDCs. These results highlight two novel TFs that regulate DC development and/or function, significantly deepening our understanding of DC biology and providing potential new targets for DC-based immunotherapies in infectious and non-infectious diseases.

Presenter: Kellar, Gerald

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

Gerald Kellar, Kathryn L. Pothoven PhD, Sabine Spath PhD, Steven F. Ziegler PhD

Benaroya Research Institute; University of Washington Immunology Department; U.S. Army Medical Department

With decreasing air quality worldwide due to manmade and natural circumstances 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 1960s, a better understanding of the infectious progression of this pathogen is critical to better understanding how to combat it. The 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 increased recruitment of C-C chemokine receptor-2 (CCR2) positive inflammatory monocytes (MO) with increased production of C-C chemokine ligand-2 when compared to 8-week old mice. CCR2+ MO have 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 CCR2+ MO in the 3-week old RSV infected mice contribute to tissue remodeling that is characteristic of the progression to asthma. Histological staining of lungs from the age-related mouse groups demonstrate that the 3-week old mice display increased accumulation of extracellular matrix (ECM) components, particularly hyaluronan and versican, both of which are traditionally increased in the asthmatic lung and can be produced and/or degraded by the MO. Additionally, the 3-week old mice have increased production of interleukin-17 and elevated lung neutrophils at 72-hours post infection, both of which have been linked to complications of RSV infection while possibly further contributing to tissue destruction. This data suggests that the immune profile of the juvenile lung could result in unproductive tissue & ECM destruction that could facilitate the conditions which support the development of asthma.

Presenter: Klawon, David E.J.

Role of a single self-peptide in the prevention of organ-specific autoimmunity

David E.J. Klawon, Dana C. Gilmore, John D. Leonard, Jaime L. Chao, Ryan K. Duncombe, Erin J. Adams, Peter A. Savage*

Department of Pathology, University of Chicago; Department of Biochemistry and Molecular Biology, University of Chicago

The display of agonist peptide ligands in the thymus, driven in part by Aire, can promote both the clonal deletion of antigen-specific T cells and the differentiation of such cells into the Treg cell lineage. Through the identification and study of T cells reactive to two natural Aire-dependent self-peptide ligands derived from the prostatic protein Tcaf3, and using gene-targeted mice lacking one of these epitopes, we define the role of cognate antigen expression in directing these alternate cell fates and preventing prostatic infiltration by antigen-specific T cells. We show that expression of a single peptide ligand in the thymus is required to direct antigen-specific monoclonal cells into the Treg lineage, and has a negligible role in driving concurrent clonal deletion. Moreover, T cell transfer experiments reveal that skewing of this single specificity to the Treg compartment is critical for the prevention of prostate-specific T cell infiltration. Thus, the protection of a peripheral organ from autoimmune attack can be dependent on T cell selection against a single self-peptide ligand.

Presenter: Kobayashi, Shio

Development of novel cell treatment method for type 1 diabetes based on CAR T-cell therapy

Shio Kobayashi, Martin Thelin, Alborz Kaimzadeh, Neha Deshpande, Heather Parrish, Mark Lee, Michael Kuhns, and Thomas Serwold

Joslin Diabetes Center

Chimeric antigen receptor (CAR) T-cell therapy is a new form of immunotherapy against cancer. A CAR is a single-module fusion protein containing a tumor-specific antigen-binding domain of an antibody, which is fused directly to signaling components of the T cell receptor (TCR) signaling pathway. CARs, when expressed by CD8+ cytotoxic T cells, confer the ability to recognize and kill target tumor cells specifically. We adapted this engineering strategy to target autoreactive T cells in a mouse model of type1 diabetes (T1D). We engineered a chimeric receptor consisting of peptide-loaded major histocompatibility complex (pMHC) fused to the proximal, and transmembrane domains of the TCRα and TCRβ chains. The pMHC-TCR fusions paired with the endogenous CD3 subunits. We also engineered a chimeric co-receptor designed to bring LCK into the signaling complex, creating a 5-module chimeric receptor (5M-CAR). 5M-CAR T-cells loaded with an MHC II-restricted autoantigen commonly targeted in the NOD mouse model of T1D, killed their target CD4 T cells in an antigen-specific manner both in vitro and in vivo. Infusion of 5M-CAR T-cells also prevented diabetes in a NOD-SCID mouse transferred with autoimmune T cells in an antigen-specific manner. These findings indicate that 5M-CAR T-cells have broad potential for elimination of harmful populations of monoclonal and oligoclonal T cells in autoimmune diseases.

Presenter: Koh, Andrew

Aire and Brg1 Define a Fulcrum for Ectopic Gene Expression in the Thymus to Provide Precise Control of Immune Tolerance

Andrew Koh, Erik Miller, Jason Buenrostro, David Moskowitz, William Greenleaf, Howard Chang, Gerald Crabtree

Stanford University

The thymus mirrors the peripheral self by expressing thousands of tissue-specific antigens to purge dangerous self-reactive clones from the T cell repertoire. How thymic epithelia gain initial access to these tissue-specific genes and activate them at levels that prevent physiologic harm is unknown. Here, we identify Brg1 as an essential determinant promoting accessibility at tissue-specific loci to poise transcription and impose central tolerance. Surprisingly, we show that Aire harbors an intrinsic repressive function that restricts chromatin accessibility and transcriptional amplitude. Aire imposes this repressive influence within minutes upon recruitment to chromatin and operates over the genome in opposition to the mSWI/SNF chromatin remodeling complex. Human autoimmune mutations that impair Aire's multimerizing and histone-binding activities also inhibit this repressive influence indicating dual roles of Aire's functional domains. Together, Brg1 and Aire define a fulcrum in thymic epithelial development that allows the access and activation of tissue-specific genes at levels that prevent toxicity, yet facilitate immunological tolerance.

Presenter: Kuan, Emma

Thymic stromal lymphopoietin promotes interplay between tumor cells, myeloid cells, and CD8+ T cells in regulation of breast cancer

Emma Kuan, Steven Ziegler

Benaroya Research Institute

A type-2 inflammation cytokine thymic stromal lymphopoietin (TSLP) recently has been shown involved in various human cancer development. In breast cancer, TSLP is reported to regulate breast tumor cells, dendritic cells, and CD4+ T cells to promote tumor progression. However, it is still largely unknown the role of TSLP in other cell types within breast cancer since TSLP receptor (TSLPR) is widely expressed on various cell types. We recently showed that breast tumor cells express functional TSLPR and myeloid cell-derived TSLP, but not tumor-derived TSLP, is the critical source to maintain tumor cell survival in vitro and in vivo. Although the lack of tumor-derived TSLP did not alter their survival in vivo, inflammatory monocytes (also called monocytic myeloid derived suppressor cells) from these mice displayed reduced suppressive function in an in vitro suppression assay. Indeed, we found inflammatory monocytes expressed TSLPR and TSLPR deficient inflammatory monocytes derived from tumor-bearing mice displayed reduced suppressive function in suppression assay. In vivo or in vitro stimulation of inflammatory monocytes with TSLP increased their suppressive function. Most importantly, conditional depletion of TSLPR only in monocytes in tumor-bearing mice resulted in reduced tumor-associated macrophages and increased expression of exhaustion marker PD-1 in tumor-infiltrating CD8+ T cells that further led to smaller primary tumors and less tumor metastasis to lungs, suggesting a pro-tumor role of TSLP in regulating monocytes in breast cancer. Interestingly, missing TSLP signaling directly in CD8+ T cells in tumor-bearing mice led to a greater primary tumor size that was due to more PD-1 expression on tumor-infiltrating CD8+ T cells, indicative of an opposite role of TSLP in CD8+ T cells in breast cancer. Our work reveals the complex regulation of TSLP in various cell populations within breast tumors and is the first to define the in vivo role of TSLP signaling in monocytes, CD8+ T cells, and breast tumor cells that can serve as a cornerstone for therapeutic intervention in breast cancer.

Presenter: Kuhlmann, Alexandra

Metabolic Adaptation of Tissue-resident Macrophages in Cancer

Alexandra Kuhlmann, Camila Robles-Otieza, Deb Ayeni, Ziyan Xu, Curtis Perry, Katerina Politi, and Susan Kaech

Yale University Department of Immunobiology

Tissue resident immune cells play a critical role in maintaining homeostasis and orchestrating host defense. Resident macrophages represent cell populations that are uniquely adapted to their specific niche, integrating multiple complex environmental cues to generate a maximally beneficial response for their client epithelia. We propose a model in which the deregulated proliferation of malignant epithelia co-opts resident macrophage homeostatic networks to favor tumorigenesis. Using a genetically inducible mouse model of lung adenocarcinoma we observed a striking expansion in the resident cells of the alveolus, the alveolar macrophage (AM). By engaging AM lipid metabolism, a metabolic program known to skew macrophages towards an M2 like state, AM homeostatic clearance of the lipid-rich alveolar surfactant aids in the maintenance of airway tolerance, critically important in the lungs where there is a high cost to inflammation. As tumors progressed, AMs up-regulated surface expression of markers associated with alternatively activated macrophages, decreased their production of inflammatory cytokines, while increasing their lipid uptake and storage. AMs had functionally altered metabolic states with tumor-associated AMs having significantly increased rates of basal respiration as well as mitochondrial uncoupling. Induction of the transcription factor PPARg is necessary for AM maturation, integrating AM surfactant clearance and consequently lipid catabolism with their tolerant immune state. Pharmacological inhibition of PPARg delayed tumor progression, reduced AM recruitment, while restoring their inflammatory cytokine production. Our data suggests that rewiring of AM metabolism, specifically via antagonism of PPARg has the potential to be a novel therapeutic target in the treatment of lung cancer.

Presenter: Laffey, Kimberly

Precocious expression and engagement of mature αβ TCR in CD4-CD8- T cell progenitors can drive NOTCH1 mutation and T-ALL transformation

Kimberly Laffey, Robert J. Stiles; Melissa Ludescher; Tessa R. Davis; Shariq S. Khwaja; Richard J. Bram; Peter J. Wettstein; Venkataraman Ramachandran; Cory E. Johnson; Richard D. Hammer; Diana Gil; Adam G. Schrum

University of Missouri

T cell lymphoblastic leukemia (T-ALL) is an aggressive cancer arising from transformation of thymocytes. Most T-ALL involves activating NOTCH1 mutations but the drivers of mutation and transformation are incompletely understood. Here we report a rare but natural oncogenically susceptible stage during T cell development in mice, and apparently in humans. Most conventional thymocytes rearrange TCRβ and TCRα loci in separate, ordered developmental stages. However, a few thymocytes in the conventional pathway rearrange both at CD4-CD8- double-negative (DN) stage. We show that such “precocious” αβ TCR expression, when engaged by self-antigens, can risk T-ALL transformation. First, ~0.01-0.05% of mouse and human thymocytes are precocious αβ TCR DN. Second, we show that both mouse and human can present T-ALL tumors with their earliest developmental phenotype matching this stage. Third, the OT1 TCR transgene is expressed in mice with parallel timing and level with the natural, TCR-precocious subset, providing a model to study antigen-dependent signaling and transformation. We show that DN OT1 thymocytes can upregulate CD69 in response to antigen presentation independent of CD8 receptor, in vitro. Furthermore, OT1 and OT1.RAG2-/- mice, but not OT1.RAG2-/-.β2M-/- mice, spontaneously develop T-ALL in vivo with activating NOTCH1 mutations, showing that MHC-I is required and pre-TCR and other signals are insufficient for oncogenesis. Transplant experiments corroborate a requirement for recipient MHC for young tumors, but a multi-passaged tumor with increased aggression lost the requirement for MHC. Finally, the earliest developmental stage identified for OT1 tumors was the precocious DN stage, in common with the human clinical case. These data together suggest that precocious TCR expression and antigen signaling can cause T-ALL tumorigenesis.

Presenter: Lau, Irene

Developing CD1d Multimers to Study Lipid-Reactive Natural Killer T Cells

Irene Lau, Taylor Sicard, Irene Lin, Tao Chan, James Lazarovits, Warren Chan, Jean-Philippe Julien, Thierry Mallevaey

University of Toronto

Natural Killer T (NKT) cells are unconventional T cells that respond to lipid antigens presented by the major histocompatibility complex (MHC) Class Ib molecule, CD1d. Two types of NKT cells exist in mammals: type I or invariant (i) and type II or diverse (d) NKT cells. Both iNKT and dNKT cells recognize a variety of mammalian and microbe-derived lipid antigens, but there is no antigen cross-reactivity between the two cell types. iNKT cells respond to lipids such as a-galactosylceramide (aGC), whereas dNKT cells recognize sulfatide. Although iNKT cells have been described to play potent immunomodulatory functions in various diseases, such as infection, autoimmunity and cancer, the biology of dNKT cells remains elusive, due to the lack of potent reagents to track them. Lipid-loaded CD1d tetramers have been successful in studies characterizing iNKT cells, but not dNKT cells, due to the low affinity T cell receptor-CD1d interaction. Thus, increasing the avidity of lipid-loaded CD1d multimers may overcome this obstacle. We have developed two types of novel multimers, taking advantage of 1) self-assembling CD1d fusion proteins, and 2) CD1d-coated gold nanoparticles (AuNPs). We show that such aGC-loaded fluorescently-labelled CD1d multimers specifically bind primary iNKT cells and iNKT cell lines. We are now comparing the staining efficiency of these CD1d multimers with conventional CD1d tetramers, and will evaluate their ability to activate iNKT cells in vitro and in vivo. In parallel, we will evaluate the ability of sulfatide-loaded CD1d multimers to stain established dNKT cell lines, and attempt to identify dNKT cells in vivo. In summary, these novel reagents may prove to be an efficient alternative to CD1d tetramers, and may ultimately allow for further elucidating the biology and functional roles of dNKT cells, and other elusive T cell populations.

Presenter: Levin, Steve

ALPN-202, a combined PD-L1/CTLA-4 antagonist and PD-L1-dependent CD28 T cell costimulator, elicits potent intratumoral T cell immunity superior to and differentiated from PD-L1 inhibitor monotherapy

Steve Levin, Ryan Swanson, Mark Maurer, Chris Navas, Chelsea Gudgeon, Kayla Susmilch, Sherri Mudri, Katherine Lewis, Stacey Dillon, Martin Wolfson, Kristine M. Swiderek, Stanford L. Peng

Alpine Immune Sciences

Background: ALPN-202 is a variant CD80 CD80 vIgD-Fc fusion protein blocking the PD-L1 and CTLA-4 checkpoints while providing PD-L1-dependent T cell activation via CD28. This strategy delivers potent T cell costimulation, which is currently missing from checkpoint inhibitor only regimens, and may be critical for the generation of clinical anti-tumor responses, seeking to broadly improve cancer outcomes. ALPN-202 has previously demonstrated preclinical anti-tumor activity superior to PD-L1 inhibition, but the specific mechanism(s) of superiority remain unreported. Methods: In a hPD-L1-transduced MC38 tumor model treated with ALPN-202 or durvalumab, an approved PD-L1 inhibitor, anti-tumor responses were evaluated by serial tumor volume measurements, and intratumoral immune responses were assessed by RNA-Seq, flow cytometry, and immunoSEQ TCR repertoire analysis (Adaptive Biotechnologies). Results: Multiple doses of ALPN-202 elicited anti-tumor responses superior to durvalumab as judged by tumor volume measurements. Efficacy was importantly also observed with single ALPN-202 doses administered intraperitoneally or intratumorally. RNA-Seq and flow cytometric analyses of tumors revealed higher T cell, NK cell, macrophage, and dendritic cell markers after ALPN-202 treatment vs. durvalumab, along with higher T cell effector transcripts, including IL-2, IFN-γ, granzyme B, and T-bet. TCR repertoire analysis demonstrated increased clonality and richness in response to ALPN-202, two characteristics previously not reported in response to PD-(L)1 or CTLA-4 inhibition alone. Conclusions: ALPN-202 elicits intratumoral immune responses superior to PD-L1 inhibition alone, including T cell infiltration and T cell effector molecules. This suggests it may translate into clinical anti-tumor responses in cancers currently resistant to checkpoint inhibition alone and/or may improve outcomes in cancers when administered in combination with existing therapies. Ongoing studies seek to further define such potential and specific clinical indications and modalities to guide upcoming clinical trials.

Presenter: Li, Shamin

Tumor-infiltrating CD39+ MAIT cells possess a Treg-like subset and bridge the gap between gut microbiome and Colorectal Cancer

Shamin Li, Yannick Simoni, Etienne Becht, Chiew Yee Loh, Bernett Lee, Teck Por Lim, Niranjan Nagarajan, Daniel Tan SW, Iain Tan BH, Evan W Newell

Fred Hutchinson Cancer Research Center

Recently, our group has shown that CD8+ tumor-infiltrating lymphocytes (TILs) are composed of bystander and tumor-specific T cells, and CD39 can be used to identify each population. Interestingly, tumor tissue is also infiltrated by a group of unconventional T cells called Mucosal-associated invariant T (MAIT) cells. Since MAIT cells recognize riboflavin metabolites produced by many species of bacteria and there is mounting evidence that gut microbiota composition can strongly influence the antitumor efficacy of drugs, we explored whether the response of these cells in tumors could be antigen-driven. The transcriptomic profiles of tumor-infiltrating MAIT cells showed an enrichment for genes related to apoptotic and TCR signaling pathways, supporting the importance of MAIT cells in tumor response. We then distinguished two different populations among tumor-infiltrating MAIT cells that are either CD39+ or CD39-, and showed that in vitro, CD39 expression is induced in a TCR-dependent manner and not by non-TCR cytokine-dependent signaling. To investigate the relationship between MAIT cells and the gut microbiome in Colorectal Cancer, we have estimated the microbial composition in each bulk tumor using whole genome sequencing data. We observed a positive correlation between CD39+ MAIT cell frequency and tumor bacterial load, suggesting that the phenotype and function of tumor-infiltrating MAIT cells could be shaped by the gut microbial composition. Lastly, using mass-cytometry to deeply profile CD39+ MAIT cells, we observed a higher expression of the inhibitory receptor CTLA-4 and identified a subset of Treg-like MAIT cells (CD4+FoxP3+) specific to the tumors. The presence of these Treg-like MAIT cells is associated with a high infiltration in the tumors of bacteria from Bacteroidetes and Fusobacteria phyla. This study has allowed to identify two distinct populations in tumor-infiltrating MAIT cells that are CD39+ and CD39-, and revealed an unreported Treg-like subset. It will not only provide new insights in the T cell tumor immune responses, but also pave the way for using MAIT cells in gut microbiome manipulations.

Presenter: Limnander, Andre

IgE plasma cells accumulate in the bone marrow during chronic allergen exposure and contribute to serological memory

Andre Limnander, Seblewongel Asrat, Navneet Kaur, Xia Liu, Li-Hong Ben, Daisuke Kajimura, Andrew J Murphy, Matthew A. Sleeman, Jamie M. Orengo

Regeneron

Immunoglobulin E (IgE) is a central player in the development and progression of allergic diseases. Allergen-specific IgE binds to FcER1 on mast cells and basophils and, when crosslinked by allergen, causes these cells to degranulate and release inflammatory mediators of the allergic response. Identifying the source of IgE serological memory is key to understanding atopic diseases and the development of novel therapies. Although not directly demonstrated, several clinical observations support the concept of IgE serological memory; 1) that allergen specific IgE is maintained in atopic patients that have not been re-exposed to allergen; 2) that allergen specific IgE can be transferred to a non-allergic recipient following bone marrow transplant; and 3) that serum IgE levels are reduced, but not abolished, in patients treated with IgE B cell/plasmablast ablation approaches (anti-membrane IgE depleting antibody). In contrast recent studies using an IgE reporter systems in mice have identified unique features of IgE-producing cells, such as the premature exit of these cells from germinal centers and their rapid development into short-lived plasma cells prone to apoptosis, leading to the hypothesis that these features make it unlikely for IgE B cells to generate high affinity antibodies, maintain cellular memory or migrate to the bone marrow to become long-lived plasma cells that sustain serological memory. However, these studies rely on high doses of antigens delivered by injection with adjuvants and therefore do not reflect the consequences of real world allergen exposure, which can be continuous or intermittent and often delivered by inhalation at low doses. In this study, we used mouse models of both acute and chronic allergic inflammation to investigate the regulation of IgE producing cells and the source of allergen-specific IgE memory. We show that antigen-independent cytokine-induced IgE class switching or short-term allergen exposure result in the generation IgE plasmablasts that mainly reside in secondary lymphoid organs, produce polyclonal IgE and do not populate the bone marrow. In contrast, we demonstrate chronic exposure to house dust mite (HDM) results in IgE plasma cells that arise from sequential class switching of IgG1 memory B cells, gradually accumulate in the bone marrow and produce IgE of sufficient affinity to support passive anaphylaxis when transferred to naive mice. Most importantly, we identify IgE plasma cells in the bone marrow of human allergic, but not healthy donors, and show that allergen-specific IgE produced by these cells can cause mast cell degranulation and anaphylaxis when transferred to naive mice that express human FcER1α. These data demonstrate that bone marrow IgE plasma cells arise during chronic allergen exposure and can establish serological memory in both mice and humans.

Presenter: Lombard-Vadnais, Felix

Characterization of the implication of the Idd2 locus in type 1 diabetes development

Felix Lombard-Vadnais, Roxanne Collin, Genevieve Chabot-Roy, Sylvie Lesage
McGill University

Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of the beta cells of the pancreas. Genetic susceptibility to T1D is conferred by complex traits. More than 20 genetic loci, termed Idd for “insulin dependent diabetes”, are linked to T1D in the NOD mice, a model which spontaneously develops T1D. The second genetic locus, namely Idd2, was identified in the 1980s, but its contribution to T1D susceptibility was never validated. We generated a NOD.B10-Chr9 congenic mouse, where the Chr9 locus encompasses part of the Idd2 locus, enabling us to assess the specific contribution of Idd2 in T1D development. Using this congenic strain, we validate that Idd2 confers resistance to insulitis and, consequently, to T1D in the NOD mouse. By generating bone marrow chimeras, we find that the resistance is conferred in a bone marrow-intrinsic manner. Moreover, we demonstrate that Idd2 contributes to the formation of germinal centers as well as the expression of the major histocompatibility complex (MHC) on B cells. Together, these data not only validate Idd2 as a genetic locus contributing to T1D susceptibility, it strongly supports recent evidence of the contribution of the humoral response in T1D susceptibility.

Presenter: Loredan , Denis

The Role of CX3CR1+ T cells in Helminth Infections

Denis Loredan , Natasha M. Girgis, P'ng Loke
New York University

CD4+ T cell help is required for immunological resistance and tolerance to parasitic helminth infections. However, the exact subsets of T cells involved and their differing roles in the immune response remain to be elucidated. The chemokine receptor CX3CR1 is expressed on monocytes, natural killer cells, and some memory and effector CD4+/CD8+ T cells respectively, and binds to a single known ligand, CX3CL1. The interaction of this receptor with its ligand has been shown to exacerbate Th2-mediated pathology in mouse modes of asthma and atopic dermatitis, although no role has been described yet for these cells in Th2-mediated immune responses to helminth infections. Using a CX3CR1-EYFP reporter mouse we have identified a subset of CD4+ T cells that are CX3CR1+ during chronic infection with Schistosoma mansoni and acute infection with Nippostrongylus brasilinesis. We have characterized these cells as activated CD44+ CD25+ ST2+ T cells present at the main sites of tissue damage, but not the secondary lymphoid organs. Our lab is making use of a CX3CR1-CreERT-IRES-YFP Rosa26-tdTomato fate-mapping mouse model to track the kinetics of this particular subset over time and determine their potential for memory cell formation. This approach has enabled us to observe a population of T cells labeled with the tdTomato fluorophore yet not actively expressing CX3CR1 in the spleens of mice infected with the rodent hookworm N. brasilinesis, indicating potential for recirculation. It remains to be determined if CX3CR1+ CD4+ T cells are a general component of type-2 immunity. As well, understanding the adaptive immune response to helminth infection can inform not just our understanding of pathology, but also attempts to develop an effective vaccine.

Presenter: Lyons-Cohen, Miranda

Examining the Role of Lymph Node Eosinophils during the Generation of Type 2 Immune Responses

Miranda Lyons-Cohen, Michael Gerner

University of Washington

Eosinophils are short-lived innate granulocytes associated with type 2 inflammation, capable of promoting protective immunity against helminth infection, while also exacerbating allergy and asthma. Eosinophils develop in the bone marrow and circulate throughout the blood, maintaining a steady state population in peripheral organs, such as the thymus, ovary, and uterus, and intestinal tissues. During type 2 inflammation, eosinophils are recruited into peripheral tissues, such as the lung, where they can mediate their effector functions. However, the presence and function of eosinophils in draining lymph nodes (LNs) during the early generation of type 2 responses has not been thoroughly investigated. Here, we have uncovered a population of SiglecF+ST2+Sirpα+GR1+ eosinophils that were present in LNs at both steady state and after immunization with a type 2 adjuvant, papain. LN eosinophils were readily identifiable by confocal microscopy and localized primarily in the outer T cell zone, T-B border, and medullary regions. Using an IL-4 mRNA reporter mouse strain, we found that LN eosinophils made copious IL-4 message and made up the majority of total IL-4 competent cells in the steady state and after papain administration. Preliminary studies using an IL-4 protein reporter strain, however, revealed that LN eosinophils did not produce detectable IL-4 protein on days 2 or 5 after papain immunization. Collectively, these studies uncover a previously unrecognized population of eosinophils in LNs that are poised for IL-4 production, suggesting a potentially important role for this immune subset in the generation or maintenance of type 2 immune responses. Determining the function, role, life cycle, and trafficking of these cells to and within the LN will be critical for understanding the mechanisms governing the generation of type 2 responses and protective immunity against parasitic helminths or during allergy and asthma.

Presenter: MacNabb, Brendan

Cross-dressed dendritic cells prime anti-tumor CD8+ T cell responses

Brendan MacNabb, Douglas E Kline, Xiufen Chen, Sravya Tumuluru, Justin Kline
University of Chicago

CD103+ dendritic cells (DCs) orchestrate anti-tumor CD8+ T cell responses by presenting tumor antigens on MHCI molecules and providing costimulatory signals in the tumor-draining lymph node (tdLN). Tumor antigen presentation is widely assumed to require classical antigen cross-presentation. However, recent studies have demonstrated that DCs can also acquire and present intact exogenous peptide:MHC (p:MHC) molecules through a phenomenon known as cross-dressing. To determine the contribution of cross-dressing and classical cross-presentation to spontaneous anti-tumor CD8+ T cell priming in vivo, the murine MHCI molecule, H2-Kb, was deleted in syngeneic B16 melanoma and C1498 leukemia cell lines. Engraftment of B16 and C1498 tumors expressing Kb-restricted model antigens in wildtype, Batf3-/-, Tap1-/-, and KbDb-/- mice has revealed three key observations: 1) Kb expression by cancer cells is required for optimal CD8+ T cell priming against Kb restricted model antigens, to the extent that there was no discernable CD8+ T cell response against Kb-deficient C1498 tumors; 2) abundant tumor-derived MHCI molecules are observed on the surface of tumor-resident DCs and macrophages; and 3) presentation of tumor-derived p:MHCI molecules by migratory DCs in the tdLN is sufficient for ex vivo CD8+ T cell priming in the absence of classical cross-presentation. Mechanisms by which DC cross-dressing occurs are being explored both in vivo and through in vitro studies of MHCI-sufficient or deficient tumor cells and bone marrow-derived DCs. In contrast with current dogma, our results identify cross-dressing as a critical mechanism of tumor antigen presentation, and a potential determinant of anti-tumor CD8+ T cell responses in mice and man.

Presenter: Maurano, Megan

A novel model of ADAR1-driven autoinflammation

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The RIG-I like receptors (RLRs) detect viral RNA and initiate the interferon response that is essential for antiviral defense. However, these pathways can be activated by our own RNA, resulting in autoimmunity. Adenosine Deaminase Acting on Double-stranded RNA (ADAR1) “edits” double-stranded RNAs (dsRNA) by converting adenosines to inosines. Editing of endogenous RNA is required to prevent the activation of the RLR MDA5, aberrant 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 one P193A allele and a frameshift or deaminase domain mutation on their second allele. The prevalence of P193A far exceeds that of any other ADAR allele in either patients or the healthy population, though no patients homozygous for P193A have been identified. P193A (P195A in mice) is in the z-alpha domain of ADAR1, whose role in ADAR1 function is unclear. To determine how P193A impairs ADAR1 function and contributes to disease, we developed a mouse model of P193A by inserting this mutation into the murine Adar locus. This new Adar P195A mouse model more faithfully recapitulates the genotype of AGS patients than current ADAR1 loss of function models, all three of which eliminate ADAR1 editing activity and require total loss of MDA5 signaling for the mice to be viable. We have found that homozygous Adar P195A/P195A mice are born at the normal Mendelian ratios. However, mice with one P195A allele combined with a null allele of either the interferon-inducible isoform of ADAR1 (p150 P195A/-) or both isoforms of ADAR1 (Adar P195A/-) have a dramatic survival defect with 100% mortality by day 120 and day 40, respectively. Prior to death, these mice are runted, with lower weights and delayed development relative to littermates of other genotypes. The survival defect and runting are MDA5 dependent: p150 P195A/- and ADAR P195A/- are partially rescued on an MDA5+/- background while mortality and runting are entirely prevented on an MDA5-/- background. Surprisingly, 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 Adar fl/P195A mice, implying that MDA5 functions other than cytokine induction are critical to the deleterious effects of ADAR1 loss of function in vivo. Future studies will focus on how the P195A mutation affects ADAR1 localization and editing of endogenous and viral RNAs.

Presenter: McDougal, Courtney E

Cyclooxygenase-2 dependent prostaglandin E2 signaling through the EP3 receptor is required for cell-mediated immunity to Listeria monocytogenes

Courtney E McDougal, Erin M Theisen, John-Demian Sauer

University of Wisconsin-Madison

Listeria monocytogenes is an intracellular bacterium currently being developed as a cancer immunotherapeutic platform due to its ability to elicit robust CD8+ T-cell responses. Though the role of cytokines in *Listeria*-stimulated immunity has been extensively studied, the influence of lipid modulators of inflammation, known as eicosanoids, is less clear. Understanding how eicosanoids impact immunity is critical as current *L. monocytogenes* based immunotherapy trials utilize modulators of eicosanoid signaling, common nonsteroidal anti-inflammatory drugs (NSAIDs), as analgesics to alleviate patient discomfort following administration of L. monocytogenes. We hypothesized that eicosanoids, particularly those produced downstream of cyclooxygenases, influence the response to *L. monocytogenes*. Use of the non-selective cyclooxygenase inhibitor indomethacin demonstrated a clear role for cyclooxygenases in *L. monocytogenes*-stimulated immunity as antigen specific T-cells and protective immunity were decreased following treatment with indomethacin. Using Cox-1-/- mice we found that cyclooxygenase-1 activity was detrimental to immunity, whereas the use of a Cox-2 selective inhibitor demonstrated that cyclooxygenase-2 activity was required for immunity. Analysis of eicosanoid levels in the spleen revealed an acute and short-lived upregulation of the eicosanoid prostaglandin E2 (PGE2) twelve hours post immunization. PGE2 induction is critical for immunity as mice deficient in microsomal PGE2 synthase-1 showed impaired cell-mediated immune responses. Furthermore, administration of PGE2 restored immunity during Cox-2 inhibition, demonstrating that it is both necessary and sufficient. Finally, preliminary data suggests that PGE2 signaling through the EP3 receptor is critical for mediating immunity, as use of an EP3 antagonist diminished cell-mediated immune responses to *L. monocytogenes*. Understanding which cells produce PGE2 following *L. monocytogenes* infection will be essential to designing optimized platforms for Listeria-based immunotherapy. In addition, determining signaling pathways induced by PGE2 during *Listeria*-stimulated immunity will be key to developing a fully optimized therapeutic platform.

Presenter: Mitchell, Patrick

PATHOGEN SENSING BY THE NLRP1 INFLAMMASOME

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

University of California, Berkeley

Inflammasomes are innate immune complexes that orchestrate downstream inflammatory signaling. Upon pathogen detection, inflammasome assembly leads to Caspase-1 dependent processing of downstream effectors (e.g., IL-1β). We have proposed that inflammasomes play a critical role in innate defense via detection of "patterns of pathogenesis:" unique, pathogen-encoded activities that distinguish pathogens from harmless microbes. Many inflammasomes sense and are subsequently activated by binding to microbial ligands, analogous to pattern recognition receptor binding of pathogen-associated molecular patterns. In contrast, the NLRP1 inflammasome is activated by proteolytic cleavage of its N-terminus. However, the mechanism by which N-terminal cleavage converts NLRP1 from its inactive to active state was unknown. Here we describe a unique mechanism of NLRP1 inflammasome activation by "functional degradation." We find that cleavage results in proteasome-mediated degradation of the N-terminal domains of NLRP1, liberating a C-terminal fragment that is a potent Caspase-1 activator. By inducing the specific degradation of NLRP1, we show that proteasome-mediated degradation of NLRP1 is not only necessary but also sufficient for NLRP1 inflammasome activation. Moreover, our new "functional degradation" model led us to identify IpaH7.8, a *Shigella flexneri* ubiquitin ligase secreted effector, as an enzyme that induces NLRP1 degradation and activation. Our results provide a unified mechanism for NLRP1 activation by diverse pathogen-encoded enzymatic activities.

Presenter: Molina, Megan

Investigating the effect of bendamustine on the STAT3-Flt3 signaling pathway in DCs

Megan Molina, Jessica Stokes, Emely Hoffman, Richard J. Simpson, Emmnauel Katsanis
University of Arizona

Our laboratory has previously demonstrated that bendamustine conditioning is a safer alternative to cyclophosphamide in an MHC-mismatched murine bone marrow transplantation (BMT) model of graft-versus-host disease (GvHD). Bendamustine is a hybrid molecule, containing alkylating groups and a purine analog, conferring unknown anti-metabolite functions. It is known to bind directly to the SH2 domain of STAT3 and inhibit its canonical signaling. STAT3 is indispensible for Flt3 Ligand (Flt3L)-dependent dendritic cell (DC) differentiation. Flt3L-derived DCs have been shown to have unique cytokine production and trafficking ability, which may have significant implications for GvHD pathogenesis. Here we sought to investigate the effect of bendamustine on host DC differentiation in vivo and in vitro. We hypothesize that by inhibiting STAT3, bendamustine triggers a positive feedback loop that promotes Flt3 signaling in host DCs. To test this hypothesis in vivo, BALB/c mice were given equivalent doses of bendamustine (40 mg/kg BEN i.v.) or cyclophosphamide (200 mg/kg CY i.p.) on day -2 before BMT. On day 0, when the conditioned recipient would normally be transplanted, pan-DCs from the recipient spleen were isolated via magnetic selection and then characterized by flow cytometry. For in vitro experiments, bone marrow was collected from BALB/c mice and cultured in the presence of Flt3L to generate bone marrow-derived DCs (BMDCs). BMDCs were cultured in the presence of increasing concentrations of bendamustine for 6 days, and LPS was added to activate DCs for the final 18 hours. Additional BMDC experiments were performed in the presence of increasing concentrations of STAT3 inhibitor (JSI-124) and Flt3 inhibitor (A220). On day 6 of culture, BMDCs were harvested for characterization. Flow cytometry was performed to measure CCR7 and Flt3 receptor (CD135) expression on the various subsets of DCs both in vivo and in vitro. Bendamustine treatment resulted in a significant increase in cDCs and significant decrease in pDCs in vivo as compared to cyclophosphamide. Bendamustine treated DCs also showed a pattern of increased CCR7 and Flt3 receptor expression, which was significant in some DC subsets. These findings advocate a potential role of Flt3L-dependent DCs in the mechanism by which BEN alters GvHD pathogenesis to limit morbidity and mortality. Thus, BEN may have advantages as an alternative agent to CY for pre-transplant conditioning in allogeneic BMT.

Presenter: Morris, Gerald

Generation of Fluorescent Protein Tagged TCRCα Mice for Identification of Dual Receptor T Cells

Gerald Morris,

University of California San Diego

The potential for a T cell to mediate its function is primarily determined by the antigenic specificity of the T cell receptor (TCR). A portion of T cells express two functional TCRs in the periphery under normal physiologic conditions due to incomplete allelic exclusion of TCRα. Despite possible important effects of dual TCR expression on the development and shaping of the T cell repertoire and potential to mediate pathologic alloreactive and autoreactive responses, studies of dual TCR cells have been limited by an inability to definitively quantify and isolate dual receptor cells. To address this, we have used CRISPR/Cas9 to generate B6 mice with eGFP (GFP) and tdTomato (RFP) fluorescent proteins linkered to the C-terminus of TCRCα. F1 mice heterozygous mice have 44.4+2.1% GFP+, 43.5+3.7% RFP+, and 11.9+3.0% GFP+RFP+T cells in the periphery. Expression of the reporters is appropriately developmentally restricted, with the appearance of GFP+, RFP+, and GFP+RFP+ cells at the double-positive thymocyte stage concomitant with CD3 expression, and single-positive CD4 and CD8 thymocytes develop comparable to wild-type B6 mice. Transgenic T cells are functional in vitro, with proliferative responses to CD3/CD28 stimulation equivalent to wild-type B6 mice. Development of these B6.TCRα-GFP/RFP mice provides a novel tool to examine the effects of dual TCR expression on T cell development and composition of functional T cell responses.

Presenter: Naradikian, Martin S

Identifying and engineering TCR specificity against solid tumor neoantigens

Martin S Naradikian, Leslie Montero, Samantha Hall, Rukman Thota, Milad Bahmanof, Luise Sternberg, Jerome Lane, Angela Frentzen, Zeynep Kosaloglu-Yalcin, Aaron Miller, Bjoern Peters, Ezra Cohen, Stephen Schoenberger

La Jolla Institute for Immunology

Neoantigens (NeoAg) offer attractive therapeutic targets for directing a patient's immune response to the immunogenic subset of mutations expressed exclusively by their cancer cells. Despite the specificity with which NeoAg enable tumor recognition, the majority of approaches for their identification rely on purely predictive methods such as calculating the ability of mutated peptides to bind to a patient's set of HLA molecules. These methods have met with limited success in revealing natural targets present on tumor cells. We have developed a novel HLA-agnostic functional approach to NeoAg identification which combines genomic sequencing with bioinformatic analysis to nominate mutations for subsequent functional analysis using patient's own T cells in an effort to identify natural responses generated under physiologic conditions. Using this, we identified a missense mutation (V205I) in the ribosomal protein RPS2 that is recognized by CD8+ T cells from tumor-infiltrating lymphocytes (TIL) of a metastatic HPV16+ Head and Neck Squamous Cell Carcinoma lesion. We then performed adoptive cellular therapy (ACT) using either unseparated TIL or those enriched for RPS2 V205I-specific CD8+ T cells and found the latter to be superior in controlling outgrowth of tumor of a PDX cell line generated from this lesion in NSG mice. Finally, we used single-cell transcriptomics to isolate the genes encoding the RPS2-specific TCR and show that it recognizes the mutated peptide bound to HLA-B*07:02. These results demonstrate that high-affinity NeoAg-specific T cell responses can be identified in cancer patients, that ACT of these cells can control tumor growth, and that the relevant TCR can be isolated for use in TCR engineering-based immunotherapy.

Presenter: Ng, Melissa

Helios predisposes human fetal CD4+ naive T cells towards regulatory T cell differentiation

Melissa Ng, Melissa S. F. Ng, Theodore L. Roth, Ventura F. Mendoza, Alexander Marson, Trevor D. Burt

University of California, San Francisco

Activation of naive CD4+ T cells by T cell receptor (TCR) stimulation and cytokine cues lead to differentiation into effector T cell populations with distinct pro-inflammatory or regulatory functions. Unlike adult naive T cells, human fetal naive CD4+ T cells uniquely differentiate into FOXP3+ regulatory T (Treg) cells upon TCR activation independent of exogenous cytokine signalling. This facility for Treg differentiation is crucial for generating tolerance in utero; however, the mechanisms underlying this fetal cell-intrinsic predisposition towards the Treg cell fate are largely unknown. Here, we reveal FOXP3-independent transcriptional and epigenetic programs shared between fetal naive T cells and committed adult Treg cells that are inactive in adult naive T cells. We show that a subset of adult Treg-specific super-enhancers is active within fetal naive T cells, including two active super-enhancers at Helios, a signature thymic Treg gene. Helios is only expressed in fetal naive T cells, but not in adult naive T cells, and only fetal-derived induced Treg (iTreg) cells continue to express Helios. Fetal, but not adult iTreg cells, have suppressed IL-2 production, which is regulated by Helios in committed Treg cells. CRISPR-Cas9 ablation of Helios in fetal naive T cells subsequently resulted in increased IL-2 production by fetal iTreg cells. Crucially, the loss of Helios expression in fetal naive T cells impaired their subsequent differentiation into Treg cells upon TCR stimulation, indicating Helios as a critical contributor to cell-intrinsic predisposition of fetal naive T cells for Treg cell differentiation. The Treg-biased transcriptional and epigenetic programs within fetal naive T cells identified here could thus be utilized to engineer enhanced iTreg populations from adult naive T cells for adoptive cellular therapies.

Presenter: Ong, SuFey

Spatially-resolved, high-plex digital profiling enables characterization of complex immune biology of the colorectal cancer tumor microenvironment

SuFey Ong, Sarah E. Church, Andrew White, Jason Reeves, Dan Zollinger, Doug Hinerfeld, Giang T. Ong, Christopher R. Merritt, Kristi Barker, Sarah Warren, Joseph M. Beechem

NanoString Technologies

Background: Spatial characterization of the tumor microenvironment (TME) interface between cancer cells, stroma and immune cells is essential for understanding tumor progression and discovering prognostic and predictive biomarkers. However, it has proven difficult to perform such studies in a highly multiplexed manner using limited sample quantity. Digital Spatial Profiling (DSP) has been developed as a research use instrument, software and chemistry for hi-plex profiling of mRNA or protein using an optical-barcode read-out. In this study, microsatellite stable (MSS) or instable (MSI) characterized colorectal tumors were characterized using DSP with 40 proteins or 48 RNA probes to evaluate active and suppressive immune mechanisms in both immune dense regions and tumor versus stroma. Methods: Sixteen FFPE colorectal tumors that were characterized for Microsatellite stability status were mounted on slides. Tissue sections were stained with a cocktail of pan-cytokeratin, CD45, CD3 and DNA fluorescent markers and 48 RNA probes or a 40 protein cocktail of antibodies conjugated to a UV-photocleavable DNA barcodes. Regions of interest (ROI) were delineated using the immunofluorescence followed by UV excitation of the defined ROIs, which releases the DNA barcodes for downstream quantitation on the NanoString Ncounter platform. Two strategies were used for selecting ROIs, 1) Geometric profiling of CD45-enriched hotspots in the tumor center and invasive margin and 2) Segment profiling of cytokeratin-positive tumor regions compared to cytokeratin-negative regions. Results: We show that deep profiling of CD45-enriched regions from the invasive margin and tumor center of MSS and MSI tumors have different immunosuppressive and activated immune phenotypes. Comparing colorectal tumors characterized as MSS, DSP was able to differentiate immune hot and cold tumors despite MSS status. Further evaluation using segment profiling of tumors versus stroma also identified specific immune proteins and RNA pathways that were distinctly related to each compartment that were different between MSI and MSS tumors. Conclusion: Our results suggests DSP has the potential to be used to predict patients' response to PD-1 immune checkpoint blockade with greater sensitivity than standard MSS/MSI profiling, and furthermore DSP may allow identification of unique localized immune characteristics that would guide combination therapeutic approaches.

Presenter: Overall, Sarah

Allosteric sensing of peptide-MHC recognition by the T cell Receptor

Sarah Overall, Andrew McShan, Kannan Natarajan, Jinasheng Jiang, Vlad Kumirov, Rui Wang, Huaying Zhao, Peter Shuck, Mulualem Tilahun, Jinfa Ying, Louise Boyd, Ad Bax, David Marguilles, Nikolaos Sgourakis

University of California Santa Cruz

The recognition of peptide-MHC (pMHC) by the TCR is a critical first step in the activation and differentiation of T cells. Mechanistically, it is still unclear how antigenic sensing by the TCR is achieved, in particular, how the TCR communicates ligand binding to CD3, which forms the entire signaling component of the TCR:CD3 complex. We have characterized perturbations of the TCR constant domains by NMR and show distinct changes upon pMHC recognition in both the alpha and beta chains, indicative of structural or dynamic changes in these regions. Perturbations in the alpha chain cluster within the hinge region between the variable and constant domains suggestive of interdomain motion following pMHC engagement. Further characterization of the bond angles within the two domains of the unliganded alpha chain further suggests that in solution, the two domains may be oriented differently relative to the crystal structure. In contrast, the beta chain shows distinct perturbations of residues E130 and T138 of the constant domain upon pMHC binding. Mutations of these residues in T cell lines inhibits receptor expression, however mutations of adjacent residues in the H3 helix of the beta chain abrogates IL-2 production, demonstrating the critical role these residues play in propagating ligand binding events from the TCR to CD3 and providing evidence that the TCR undergoes allosteric changes upon pMHC recognition.

Presenter: Pothoven, Kathryn

Airway epithelium from asthmatic patients have an elevated immune response to human rhinovirus infection compared to healthy patients

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

Benaroya Research Institute

Human rhinovirus (HRV) infection is a common viral trigger of asthma exacerbation. Airway epithelial cells express ICAM1, the entry receptor for most strains of HRV and 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 infection in asthma patients is altered compared to control patients. Airway epithelial cells were obtained from control and asthmatic pediatric patients, grown at air-liquid interface (ALI) and left uninfected or infected with HRV16. RNAseq was performed on the samples and the data was further analyzed using an algorithm assigning differentially regulated genes to modules of genes that all have similar expression patterns. The collective gene expression of these modules were then compared between media control and HRV infection in both healthy and asthmatic patients. Two modules were identified to be elevated in ALI cultures derived from asthmatic patients upon HRV infection compared to ALI cultures from healthy patients. Gene ontology (GO) analysis identified that the first module was comprised of genes associated with type 1 interferon (IFN) signaling, response to IFNβ, negative regulation of viral genome replication and IFNγ signaling, indicating that the epithelium from asthmatic patients had an elevated antiviral immune response compared to controls upon infection. GO analysis also identified that the second module was comprised of genes associated with antigen presentation and processing via MHC class I, leukocyte and neutrophil chemotaxis, and I-κB kinase and NF-κB signaling that was elevated in ALI cultures from asthmatic patients compared to ALI cultures from healthy patients. These data suggest that epithelia from asthmatic patients have an elevated immune response to HRV compared to controls which may contribute to the development of subsequent asthma exacerbations.

Presenter: Prahl, Mary

The Impact of the Carbamate Pesticide Bendiocarb on the Fetal and Maternal Immune System

Mary Prahl, Odorizzi P, Gingrich D, McIntyre TI, Budker R, Farrington L, Jagannathan P, Wamala S, Nalubega M, Musinguzi K, Naluwu K, Sikyoma E, Kakuru A, Havlir DV, Kamya MR, Aweeka F, Dorsey G, Feeney M

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Pregnancy is a particularly high-risk period in which environmental exposures may impact the developing fetus, with uncertain long-term consequences. Prenatal pesticide exposure has been linked with numerous adverse birth outcomes, adverse neurologic outcomes, and adverse respiratory symptoms. Despite bendiocarb being a WHO approved pesticide for household indoor residual spraying (IRS) for vector control, the effects of prenatal exposure to this pesticide are understudied. Numerous pesticides including organophosphates and carbamates have been described to be immunotoxic, with biologic alterations in T cells, B cells, and cytokines. Despite the widespread use of the carbamate pesticide, bendiocarb, internationally, the immunologic effects of this pesticide have not been investigated. Prior studies of the biologic consequences of pesticide exposure have been limited by heterogeneity of the exposure with no information as to the magnitude of exposure based on blood concentration measurements in the populations most affected. We had the unique opportunity to rigorously evaluate the effect of pesticide exposure during pregnancy on the fetus. During a recent prenatal birth cohort (NICHD PROMOTE-II) in Tororo, Uganda, 300 pregnant women and their subsequent infants were enrolled in a malaria study. The initial aim of the study was to evaluate the effect of prenatal malaria exposure on the fetal immune system. However, half way through the study period, the government of Uganda commenced household indoor residual spraying with bendiocarb. Of the study participants, 60% were exposed prenatally to bendiocarb between 19 to 41 weeks of pregnancy. Using mass spectrometry, we found that at the time of delivery, 84% of IRS exposed mothers had detectable bendiocarb in their plasma. We then determined the transplacental transfer of the pesticide to the fetus, and found that 47% of exposed infants had detectable bendiocarb in cord plasma that directly correlated to maternal levels ($p<0.0001$). Remarkably, we found that the magnitude of bendiocarb exposure in the pregnant women based on maternal plasma concentrations is directly associated with changes in the fetal immune system. Higher maternal bendiocarb levels were associated with lower frequencies of cord blood Tregs ($p=0.010$, $Rs= -0.247$). In addition, higher maternal bendiocarb plasma concentrations are associated with increased mitogen stimulated (PMA/io) cord blood non-naïve T cell cytokine production of CD4+IL-2 ($p=0.004$, $Rs=0.372$), CD4+IL-8 ($p=0.009$, $Rs=0.383$), and CD8+IL-2 ($p=0.009$, $Rs=0.336$). Additionally, when we evaluated all infants that were prenatally exposed to bendiocarb, any exposure during pregnancy, we found that the frequency of cord blood T regulatory cells (Tregs) were significantly lower in infants whose mothers were exposed to IRS prenatally ($p=0.0025$). Additionally, we found further alterations in immune parameters including: increased production of inflammatory cytokines IL-2, TNF, and IL-8 during mitogen stimulation of CD4 and CD8 cord blood T cells, increased cord plasma cytokines IL-2, IL-5, IL-7, MDC, TNF-RI using multiplex bead assays, decreased cord blood T cell proliferation using CFSE assays, decreased absolute counts of whole cord blood dendritic cells, and maternal T cell proliferation in those prenatally exposed to bendiocarb. Alterations in the fetal immune system remained significant even after adjustment for prenatal malaria exposure, maternal malaria chemoprevention, infant sex, and CMV co-infection. Overall, our findings indicate that prenatal pesticide exposure with bendiocarb directly impacts the fetal immune system and skews immune responses away from a regulatory response to an inflammatory effector response.

Presenter: Proekt, Irina

Antigen presentation by thymic stromal cells enforces central tolerance

Irina Proekt, Corey Miller, Mark Anderson

UCSF

Abnormal selection of self-reactive T cells in the thymus is a key step to initiation of autoimmune disease. During development, single positive CD4+ T cells are exposed to a wide array of tissue-specific antigens (TSAs), which are produced by thymic medullary epithelial cells (mTECs) under the control of Autoimmune Regulator (Aire). TSAs can be presented in the context of major histocompatibility complex class II molecules (MHCIIs) by either mTECs or thymic dendritic cells. T cells that recognize these TSA-MHCII complexes are either deleted or positively selected to become regulatory T cells (Tregs). Although Aire+ mTECs express high levels of MHCII, the role of direct presentation to CD4+ thymocytes by these cells is not well understood. To address that, our lab has created a mouse model that allows for inducible genetic ablation of MHCII specifically in the Aire-expressing mTEC subset by using a tamoxifen-inducible Cre recombinase under the control of the endogenous Aire locus (iAire-cre) in combination with mice homozygous for an MHC Class II locus flanked by loxP sites (MHCIIfl/fl). We have shown that this model allows for efficient and specific ablation of MHCII from the surface of mTECs without affecting other thymic antigen-presenting cell subsets or perturbing thymocyte development. In addition, ablation of MHCII expression on mTECs completely disrupts negative selection and Treg conversion in the Rip-mOVA x OTII model. Furthermore, lack of MHCII on mTECs leads to loss of peripheral tolerance to an endogenous Aire-regulated retinal antigen, IRBP. These data suggest that direct presentation of self-antigens by mTECs is a major tolerance mechanism during thymic T-cell development.

Presenter: Rackaityte, Elze

Bacteria in human intestine promote mucosal immune development in utero

*Elze Rackaityte, Rackaityte E, Halkias J, Fukui EM, Mendoza VF, Crawford ED, Fujimura KE, Burt TD, Lynch SV**

UCSF

Mucosal immunity influences host-microbial interactions and is evident in the human fetal intestine by 11-14 weeks of gestation; the developing intestine is populated by lymphoid aggregates, memory T cells and dendritic cells capable of responding to microbial stimuli. Recent evidence for bacterial presence in utero comes from DNA-based, culture-independent studies of the placenta and neonatal meconium, the latter comprised of amniotic fluid ingested during gestation. However, whether intestinal encounters with viable microbes occur in utero and shape immune maturation has not been investigated. Here, using culture-independent methods, we identified subsets of fetal meconium relatively enriched for *Lactobacillus* or *Micrococcus* that related to divergent epithelial cell layer transcriptomes and proportions of lamina propria innate-like PLZF+ CD161+ CD4+ T cells in paired intestinal samples. Mimicking conditions in the fetal intestine permitted isolation of viable Lactobacillus and Micrococcus strains from fetal meconium and, in contrast with phylogenetically related reference strains, fetal isolates utilized placental hormones for growth, remained viable within macrophages, and exhibited species-specific capacity to promote immune tolerance. Fetal *Lactobacillus* isolates reduced activation of antigen presenting cells and promoted IL17F production in T cell co-cultures; the *Micrococcus* isolate reduced TNFα production in antigen presenting cells and inhibited IFNγ production by memory PLZF+ T cells. Whole genome sequencing of fetal isolates identified them as strains of *Micrococcus luteus* or *Lactobacillus jensenii* and phylogenetically unique genes for intracellular survival, immune modulation, and steroid uptake and metabolism were identified. These data suggest that pre-natal immunity is influenced by bacterial exposure in the fetal intestine, identifying a previously unknown component of human mucosal immune development.

Presenter: Radu, Caius

Constitutive Interferon signaling triggers ATR and PARP dependency in pancreatic cancer

Caius Radu, Evan Abt, Shili Xu, Amanda Maas, Thuc Le, Joe Capri, Soumya Poddar, David Dawson, Timothy Donahue

University of California, Los Angeles

Pancreatic ductal adenocarcinoma (PDAC) tumors are defined by high rates of KRAS mutations, a dense stromal compartment and an inflammatory microenvironment. Here we show that a subset of PDAC tumors exhibit constitutive type I interferon (IFN) signaling as indicated by gene expression and immunohistochemistry analyses. Constitutive IFN signaling also occurs in PDAC patient-derived and cell line models, and requires an active cGAS/STING pathway. Using mass spectrometry phosphoproteomic and metabolic analyses, and chemical genomics high-throughput screening approaches we determined that IFN signaling triggers addiction to the replication stress response pathway. Inhibition of ATR kinase, the master regulator of the replication stress response pathway, induces extensive reprogramming of nucleotide metabolism and triggers replication catastrophe in in PDAC cells with constitutive IFN signaling. Pharmacological PARP inhibition further sensitizes these cells to ATR inhibition. Collectively, this work begins to define the crosstalk between pro-inflammatory cytokine signaling, DNA replication/damage stress response networks and nucleotide metabolism in PDAC, and provides the rationale for new clinically applicable therapeutic approaches.

Presenter: Redford, Samuel

Adipose tissue physiology in host-parasite interactions

Samuel Redford, Samuel Redford, Janelle Ayres

Salk Institute, UCSD

Traditionally, white adipose tissue was believed to function solely as an energy storage tissue, however, evidence from recent studies have shown WAT is a critical regulator of immune responses. This is best appreciated in the context of metabolic diseases where the morbidities such as hyperinsulinemia, hyperglycemia, and chronic low-grade inflammation, can be associated with improper immune activation within the WAT. A role for adipose tissue during infections is less well understood. Multiple pathogens have been found to localize in adipose tissue during chronic infection including parasites, bacteria, and viruses. We are interested in why pathogens localize to the adipose tissue for a chronic infection. To address our goal, we are utilizing the eukaryotic, single cellular parasite, *Trypanosoma brucei. T. brucei* is the causative agent of African sleeping sickness in humans and trypanosomiasis in animals. The parasite has previously been thought to mainly reside in the blood before entering the central nervous system before causing death. However, it has recently been found to localize to adipose tissue and has more parasites in the adipose tissue than in any other tissue, including blood, once the disease enters the chronic phase. Here I describe our initial studies characterizing the host metabolic responses to *T. brucei* over the course of the infection.

Presenter: Reiner, Gabrielle

ADU-S100 (MIW815) Combines with Checkpoint Inhibition to Elicit an Anti-Tumor CD8+ T Cell Response to Control Distal Tumors

Gabrielle Reiner, Kelsey Sivick Gauthier[1], Anthony L. Desbien[1], Leticia Corrales[1], Weiwen Deng[1], Natalie H. Surh[1], Brian Francica[1], Justin J. Leong[1], Ken Metchette[1], Lianxing Zheng[2], Charles Cho[3], Yan Feng[2], Jeffrey M. McKenna[2], Steven L. Bender[3], Chudi Ndubaku[1], Meredith L. Leong[1], Andrea van Elsas[4], and Sarah M. McWhirter[1]

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STimulator of Interferon Genes (STING) is a critical component of an innate immune pathway that activates robust anti-viral and anti-tumor responses in mouse models. Small molecule agonists of STING are being developed as cancer immunotherapeutics due to potent anti-tumor efficacy and induction of immunity to rechallenge in pre-clinical models. Activation of the STING pathway by intratumoral (IT) injection of synthetic cyclic dinucleotides (CDNs) induces type I interferons in tumor resident-myeloid subsets, activation of antigen presenting cells, expansion of tumor-specific CD8+ T cells and control of tumors. In this study, we explored the benefit of combining CDN IT therapy with immune checkpoint blockade. ADU-S100 (MIW815), a CDN under clinical evaluation, was administered by IT injection in syngeneic mouse tumor models to assess the efficacy in combination with checkpoint inhibition. In mice bearing dual flank 4T1 mammary carcinoma tumors resistant to anti-PD-1 treatment, adding a single dose of ADU-S100 with anti-PD-1 induced eradication of both injected and non-injected tumors, leading to complete responses, demonstrating that ADU-S100 potentiates the activity of checkpoint blockade. Tumor control was CD8+ T cell-dependent and correlated with an enhanced CD8+ T cell effector profile in both the periphery and in non-injected tumors. Combining a single injection of ADU-S100 with anti-PD-1 also elicited enhanced tumor control in the MC-38 colon carcinoma model compared to ADU-S100 or anti-PD-1 treatment alone. Moreover, in the poorly immunogenic B16.F10 model, adding ADU-S100 to the ineffective combination therapy of anti-PD-1 and anti-CTLA-4 induced tumor-specific CD8+ T cell responses and tumor control, leading to multiple complete responses and durable immunity in surviving animals. Together, these results highlight the immune correlates of STING-mediated anti-tumor efficacy and illustrate the potential of combining ADU-S100 (MIW815) with checkpoint inhibitors for the treatment of human cancer. Clinical trials of ADU-S100 in combination with anti-PD-1 or with anti-CTLA-4 are ongoing and could further elucidate the immunological mechanism of action and therapeutic effect in humans.

Presenter: Roberts, Lydia

Pulmonary resident effector CD4+ T cells fail to effectively utilize glycolysis

Lydia Roberts, Tyler D Evans, Catharine M Bosio

Rocky Mountain Laboratories, NIAID, NIH

During the transition of T cells from naive to effector cells, they undergo a metabolic shift from oxidative phosphorylation to glycolysis. The ability of effector cells to undergo aerobic glycolysis is thought to be an essential process for the production of effector molecules such as IFN-γ and granzyme B. Vaccination or infection within the pulmonary compartment establishes a pool of lung-resident, effector memory T cells (Tem). In many cases, this pool of resident Tem cells is sufficient to control a secondary infection. Therefore, we hypothesized tissue-resident Tem would be highly glycolytic upon activation. To test this hypothesis, we utilized intranasal vaccination with two pulmonary bacterial pathogens, Francisella tularensis and Bordetella pertussis, to elicit tissue-resident CD4+ Tem cells. Surprisingly, following real-time activation of purified cells with anti-CD3/CD28 beads in the Seahorse Bioanalyzer, tissue resident CD4+ Tem cells did not shift to glycolysis whereas circulating CD4+ Tem cells became highly glycolytic. Tissue resident CD4+ Tem also did not increase mitochondrial respiration as a mechanism to compensate for the absence of glycolysis. Addition of metabolites or cytokines to further activate tissue resident CD4+ T cells did not significantly alter their metabolic profile. Together these data suggested that, unlike circulating CD4+ T cells and conventional splenic T cells, tissue resident CD4+ Tem are metabolically quiescent ex vivo. Importantly, these results were similar for both pathogens indicating that the intrinsic differences in pulmonary T cell populations is independent of infecting agent. While these results were unexpected based on current dogma regarding T cell metabolism, the lung is a unique environment compared to commonly studied lymphoid organs. The requirement to appropriately temper inflammation to maintain pulmonary function suggests lung parenchymal T cells behave differently than T cells within other organs, i.e the spleen, or those in circulation. Furthermore, our data suggest there are dynamic inflammatory changes within the pulmonary compartment in vivo that are essential to provoke optimal resident T cell metabolism and subsequent immunity.

Presenter: Robinson, Elektra

Characterization of an Inflammatory Specific Isoform of Aim2 that is Regulated by Iron

Elektra Robinson, Sergio Covarrubias, Barbara Shapleigh, Rojin Safavi, Ran Song, Edward K Wakeland, Mark Akeson, Susan Carpenter

UC Santa Cruz

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, that once activate initiate complex signaling cascades resulting in induction of an inflammatory program. Perturbations to these signaling pathways can have devastating consequences, leading to autoimmune diseases and cancer. An inducible gene expression program is a critical component of the host inflammatory response. The protein Aim2 (Absent in Melanoma) is the cytosolic receptor for dsDNA within the cytosol. Once Aim2 is activated by DNA it results in the formation of the inflammasome complex leading to the production of the proinflammatory cytokines Il1b and IL18. From a combination of high throughput approaches including ChIP-seq, ATAC-seq and RNA-seq we identified an active promoter and transcript 1kb upstream of the annotated promoter and transcription start site (TSS) for Aim2. Interestingly this region is more active following inflammatory activation compared with the canonical TSS. Initially we investigated this region as a possible long noncoding RNA but following direct RNA-sequencing of macrophages before and after inflammatory activation using Oxford Nanopore we found that this region is in fact an alternative 1st exon for Aim2. Long read sequencing provided us with the essential isoform information from this locus, something that was missed with our previous approaches. Our functional data G. indicates that this inflammatory activated isoform is translated at a lower level compared to the canonical mRNA isoform. Translation is controlled through an iron response element (IRE) that is present within the alternative 1st exon. Using reporter assays we confirm that indeed the IRE is the critical element controlling the translational efficiency as removal of this element or treating the cells with iron reverses this effect. This novel mRNA isoform of Aim2 is conserved between human and mice. Future work will aim to uncover the importance of this alternative isoform switch in the context of human diseass such as Systemic Lupus Erythematosus.

Presenter: Romero, Alicia

Muscle wasting is a maladaptive host metabolic response to infection

Alicia Romero, Alicia Romero, Janelle Ayres

University of California San Diego

During infection, hosts endure significant metabolic changes that can dramatically influence host defense responses and pathogen behavior. Muscle wasting is one of the most highly conserved metabolic changes observed across a wide range of infections and across the animal kingdom. The function of muscle wasting during infection, if any, is not understood. It has been proposed that muscle wasting is a host metabolic response that modulates energy allocation during infection. However, current experimental and clinical evidence show that muscle wasting decreases host survival and impairs recovery from severe infections. Using a transgenic murine model lacking a critical mediator of muscle atrophy (FoxO1) in skeletal muscle, we will determine why and how muscle wasting leads to a more severe outcome of infection in the context of *Salmonella Typhimurium* infection. Mice deficient for muscle Foxo1 had increased survival when challenged with a lethal dose of *S. Typhimurium*. Consistent with our previous report, we found that the protection from muscle wasting was not accompanied by changes in pathogen burden. This suggests that muscle wasting increases disease severity by promoting pathogen virulence or impairing a disease tolerance mechanism rather than host resistance.

Presenter: Roy Chowdhury, Roshni

Integrative multi-cohort systems analysis reveals immune determinants of M. tuberculosis infection outcomes and treatment response

Roshni Roy Chowdhury, Francesco Vallania, Qianting Yang, Cesar Joel Lopez Angel, Fatoumatta Darboe, Adam Penn-Nicholson, Virginie Rozot, Elisa Nemes, Stephanus T. Malherbe, Katharina Ronacher, Gerhard Walzl, Willem Hanekom, Mark M. Davis, Jill Winter, Xinchun Chen, Thomas J. Scriba, Purvesh Khatri and Yueh-hsiu Chien

Stanford University

One-quarter of the global population is infected with *Mycobacterium tuberculosis* (Mtb). Approximately 90% of these individuals successfully contain the infection and manifest a clinically asymptomatic state, called latent tuberculosis infection. Although only 10% progress to active disease, tuberculosis (TB) is a leading cause of death worldwide. Despite intense efforts, the immune factors that determine these outcomes, and the mechanisms that underlie the transition from latency to active TB remain largely unknown. Therefore, a better understanding of the "immune profiles" associated with disease progression, resolution and long-term outcomes is critical for the identification of intervention points and the development of new therapies. To address these knowledge gaps, we developed an integrative framework by incorporating multi-dimensional mass cytometry (CyTOF), serum proteomics, functional assays and cellular deconvolution of transcriptomic data to investigate: (1) the immune state associated with TB latency and (2) how it changes with disease progression and treatment response. Through the analysis of a cohort of South African adolescents, we found that latent tuberculosis is associated with enhanced cytotoxic responses, mostly mediated by CD16 (also known as FcγRIIIa) and natural killer (NK) cells, and continuous inflammation coupled with dysfunctional T and B cell compartments. Additionally, we showed that irrespective of age, genetic background and geographic location, peripheral NK cell levels were a discerning cellular biomarker for TB disease states in global cohorts. Changes in circulating NK cell levels could predict disease progression, treatment response and reflect the activity level and burden of Mtb infection in a patient's lungs. These findings offer critical insights into the underlying pathophysiology of TB latency, identify factors that may influence infection outcomes, and have the potential to change the course of TB diagnosis and prevention.

Presenter: Ruterbusch, Mikel

Infection with respiratory syncytial virus drives an enhanced allergen-specific Th2 response following subsequent house dust mite exposure

Mikel Ruterbusch, Marion Pepper

University of Washington

Allergic asthma is a global health burden, affecting over 300 million individuals worldwide. Even with treatment, these individuals are at risk of hospitalization and death from acute asthma-related complications. Improving our understanding of how allergic asthma develops and is maintained is essential to further treat and prevent this disease with novel therapeutic and environmental interventions. Among several other risk factors, children are more likely to develop atopic asthma throughout the remainder of childhood if they contract a severe respiratory syncytial virus (RSV) infection during infancy. However, it is still unclear how severe RSV infections are linked to the development and exacerbation of allergic asthma long after the virus has cleared. We have identified that in contrast to influenza A virus, intranasal infection with RSV drives the production of viral-specific CD4+ T cells in the lung that produce the type 2 cytokines IL-13 and IL-5 at eight days post-infection. Furthermore, we have found that compared to mice treated with house dust mite (HDM) alone, mice infected with RSV three weeks prior to allergic sensitization with HDM possess more Th2-differentiated CD4+ T cells specific for the HDM-derived protein Der p 1 in the lung early after allergic challenge. Our findings suggest that infection with RSV can induce viral-specific CD4+ Th2 cells and leads to increased numbers of Der p 1-specific CD4+ Th2 cells in the lung upon subsequent allergic sensitization and challenge with HDM. We are currently investigating the mechanisms underlying these observations to improve our understanding of how RSV infection impacts the development of asthma, which may inform new therapeutic strategies to treat or prevent allergic asthmatic disease.

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

Many immune responses are initiated through the recognition of pathogen-associated molecular patterns (PAMPs), such as flagellin or bacterial lipopolysaccharide, by germline-encoded receptors. While the mechanisms by which these receptors recognize PAMPs are well established, there is mounting evidence that the immune system is also able to recognize pathogen-associate activities, such as pathogen-induced potassium efflux or pathogen-associated enzymatic activities. However, the molecular mechanisms by which the immune system can detect these pathogen-associated activities are unclear. To uncover such mechanisms, we investigated how the NLRP1B inflammasome is able to sense the enzymatic activity of the *Bacillus anthracis* lethal factor (LF) protease. Unexpectedly, we found that cleavage of NLRP1B by LF induces proteasomal degradation of NLRP1B. Degradation of NLRP1B releases an active fragment of NLRP1B which self-oligomerizes, and recruits and activates caspase-1 to initiate a downstream inflammatory response. This model of activation further led us to identify a novel activator of NLRP1B, the secreted E3 ligase IpaH7.8 from *Shigella flexneri*. These results identify a novel unified mechanism through which the NLRP1B inflammasome is able to detect pathogen-associated enzymatic activities.

Presenter: Sgourakis, Nikolaos

Chaperone-assisted peptide exchange on MHC-I is driven by a negative allostery release cycle

Nikolaos Sgourakis, Andrew C. McShan, Kannan Natarajan, Sarah A. Overall, Vlad K. Kumirov, David Flores-Solis, Jiansheng Jiang, Mareike Badstuebner, Jugmohit S. Toor, Danai K. Moschidi, Clive R. Bagshaw, Evgenii L. Kovrigin, David H. Margulies, and Nikolaos G. Sgourakis

University of California, Santa Cruz

Molecular chaperones TAPBPR (TAP-binding protein related) and tapasin associate with class-I major histocompatibility complex (MHC-I) molecules to promote optimization (editing) of peptide cargo. Here, we use methyl-based, solution NMR to investigate the molecular mechanism of peptide exchange performed by the 90 kDa chaperone protein complex. We identify TAPBPR-induced conformational changes on conserved MHC-I surfaces, consistent with our independently determined X-ray structure of the peptide-deficient complex. Conformational dynamics present in the empty MHC-I are stabilized by TAPBPR in a peptide-deficient complex, and become progressively dampened with increasing peptide occupancy. Incoming peptides are recognized by the chaperoned groove according to the global stability of the final pMHC-I product, and anneal in a native-like conformation. Our results demonstrate an inverse relationship between MHC-I occupancy by peptide and the affinity of TAPBPR for such pMHC-I molecules, where the lifetime of transiently bound peptides controls the dynamic regulation of a conformational switch, located near the TAPBPR binding site, which triggers TAPBPR release. Lastly, we discuss the role of protein dynamics in shaping chaperone specificity towards different human and murine class-I MHC alleles. These results suggest a similar mechanism for the editing function of tapasin in the peptide-loading complex.

Presenter: Simoni, Yannick

Identification of tumor-specific CD8+ T cells in EBV driven lymphoepihtelioma-like carcinoma: a case report

Yannick SIMONI, Etienne BECHT, Chiew Yee LOH, Joe Poh Sheng YEONG, Tony Kiat Hon LIM, Angela TAKANO, Daniel S.W. TAN, NEWELL W. Evan

Fred Hutchinson Cancer Research Center

Lymphoepithelioma-like carcinoma (LELC) of the lung is an uncommon lung cancer, typically observed in young, non-smoking Asians populations. LELC is associated with the presence of Epstein-Barr virus (EBV) in lung tumor tissue, suggesting the carcinogenic role of EBV as observed in nasopharyngeal carcinoma. Here, we studied the antigen specificity and phenotype of tumor infiltrating (TIL) CD8+ T cells in one LELC patient positive for EBV infection in lung tumor cells. Using an MHC class I tetramer, we detected two populations of EBV-specific CD8+ TILs, which can be considered as tumor-specific CD8+ T cells, in the tumor of this patient. Transcriptomic analyses of these two populations reveal their distinct exhausted phenotypes and their polyclonal TCR repertoire. High dimensional analyses at protein level utilizing mass cytometry show the heterogeneity of each tumor-specific CD8+ TIL in the tumor. More importantly, it has been found that LELC tumor cells express PD-L1; however, tumor-specific CD8+ T cells do not necessarily express PD-1, suggesting that immunotherapy (i.e. anti-PD-1 treatment) may not be an appropriate strategy for treating LELC patients.

Presenter: Singh, Brenal

Allergic airway inflammation and airway hyperresponsiveness are independently controlled by diacylglycerol kinase zeta

Brenal Singh, Wen Lu, Amanda M. Schmidt Paustian, Moyar Q. Ge, Cynthia J. Koziol-White, Cameron H. Flayer, Sara S. Killingbeck, Nadan Wang, Xinzhong Dong, Matthew J. Riese, Deepak A. Deshpande, Reynold A. Panettieri, Jr., Angela Haczku, and Taku Kambayashi

University of Pennsylvania

Asthma is a chronic allergic inflammatory airway disease caused by aberrant immune responses to inhaled allergens, leading to airway obstruction caused by airway hyperresponsiveness (AHR) to contractile agonists. Here, we report that targeting diacylglycerol (DAG) kinase zeta (DGKζ), a negative regulator of DAG-mediated cell signaling, protects against allergic asthma by simultaneously blocking airway inflammation and AHR by independent mechanisms. Targeted deletion of DGKζ in T cells led to decreased type 2 inflammation in an ERK-dependent manner with no attenuation of AHR. In contrast, loss of DGKζ in airway smooth muscle cells led to decreased AHR in an ERK-independent manner despite no changes in airway inflammation. Importantly, pharmacological inhibition of DGK diminished airway inflammation and AHR in mice, and also reduced bronchoconstriction of human airways. These data suggest that DGK is a novel therapeutic target for asthma and reveals that the inflammatory and AHR components of asthma are not as interdependent as generally believed.

Presenter: Stolley, Michael

Ontogeny and function of "Trm-like" CD8 T cells within the mediastinal lymph node following influenza virus infection.

Michael Stolley, David Masopust

University of Minnesota

Following influenza virus infection, we unexpectedly observed a sizable population of memory T cells within the lung-draining lymph node (medLN) expressing canonical markers of tissue-residency including CD69, CD103, and granzyme B; closely resembling tissue resident memory (Trm) T cells from the upstream tissue. Moreover, these "Trm-like" cells were greatly enriched in the medLN compared to other non-draining lymph nodes suggesting disequilibrium with the circulation. Among its nuanced functions, CD69 is transiently upregulated upon TCR stimulation. Accordingly, previous reports have attributed the presence of CD69+ memory T cells in the medLN after pulmonary infection to tonic TCR signaling from a "depot" of residual antigen. Arguing against this interpretation, we failed to observe proliferation or conversion to a "Trm-like" phenotype of congenically distinct antigen-specific T cells adoptively transferred into influenza-memory mice 30 days post-infection. Additionally, inflammation alone was insufficient to drive a "Trm-like" phenotype on bystander LCMV-specific memory T cells when mice were infected with influenza virus expressing an irrelevant antigen. Given these observations, we instead favored the hypothesis that "Trm-like" cells populating the medLN emigrated directly from the lung following resolution of pulmonary infection. To test this hypothesis, a depletion strategy was utilized by which we could eliminate circulating influenza-specific memory CD8 T cells while preserving those within the airways and lung parenchyma. At an early timepoint post-depletion, flu-specific memory T cells were efficiently removed from blood and secondary lymphoid tissues including the medLN. Strikingly, a population of memory T cells re-emerged within the medLN after 30+ days post-depletion where they phenocopied cells from the lung parenchyma. Functionally, "Trm-like" memory T cells from the medLN more rapidly reacquired a Trm phenotype upon entry into the lungs and gut after secondary challenge with influenza virus (but not LCMV) infection. Teleologically, the lung is a sensitive tissue primarily involved in gas exchange where immunity is known to rapidly wane. We propose a model by which pulmonary immunosurveillance is achieved, while avoiding potentially deleterious consequences of persistent lung memory, through the stockpiling of resident memory T cells in the draining lymph node.

Presenter: Stuart, Sara

Mechanisms of Activation- Induced Chromatin Decondensation in the Lymphoid Lineage

Sara Stuart, Jeremy Irsik, Jamila Johnson, Ryan Scruggs, Hailey Sobota, Chelsea Joseph, Morgan Fretwell, Sierra McDonald, Alynna Knaub, and Jason Rawlings

Furman University

During an immune response, αβ T cells undergo a dramatic change in chromatin configuration, from a very condensed organization seen in naive cells to a decondensed state in activated cells. It has previously been demonstrated that this change in chromatin architecture is required for αβ T cell proliferation. Since B cells, γδ T cells, and NK cells share the same lineage as αβ T cells, originating from a common lymphoid progenitor, we hypothesized that chromatin status could regulate their proliferation as well. Using flow cytometry, we analyzed chromatin status as a function of activation and found that in their basal (naive) state, each cell type of the lymphoid lineage experiences a different state of chromatin condensation, which correlates with their known ability to proliferate in response to activation stimuli: the chromatin in both NK cells and γδ T cells is more decondensed than αβ T cells while B cells have similarly condensed chromatin. We then tracked chromatin decondensation during activation and found that B cells and αβ T cells decondensed chromatin in a similar manner, although there appears to be differences in kinetics between the two. NK cells, however, experience a lesser rate of decondensation compared to αβ T cells. Finally, we identified two distinct populations of γδ T cells based on Thy1.2 staining. Thy1.2hi (naive) decondensed chromatin similarly to αβ T cells, whereas Thy1.2lo (memory) γδ T cells decondensed chromatin to a greater extent than αβ T cells. Previous work in the lab demonstrated that calcium regulates chromatin decondensation in αβ T cells, suggesting that other lymphocytes could utilize a similar mechanism for decondensation. Using BAPTA, a known calcium chelator, we show that calcium is required for proper chromatin decondensation in B cells. Future studies will investigate if calcium can also regulate the decondensation of γδ T cells and NK cells, establishing a conserved mechanism to control proliferation of the lymphoid lineage.

Presenter: Tantin, Dean

Genetic and pharmaceutical targeting of the Oct1-OCA-B pathway protects against autoimmunity while preserving beneficial immune function

Dean Tantin, Heejoo Kim, Arvind Shakya, Laura Dickey, Colleen Stone, Jillian L. Jafek, Danny Chou, Thomas E. Lane

University of Utah Department of Pathology, Division of Microbiology & Immunology

The development of new treatments to block pathogenic autoimmune responses while keeping beneficial immune function largely intact constitutes a major goal in the field. The transcriptional co-regulator OCA-B is induced in stimulated primary CD4 T cells, where it docks with its cognate transcription factor Oct1 to directly recognize and regulate genes such as Il2, Il21, Ifng, Icos and Csf2 (Gmcsf). We have shown that Oct1 and OCA-B only regulate these targets in cases of antigen reencounter. For example, both proteins are dispensable for expression of these genes upon simple stimulation of CD4 T cells. This dispensability forms part of a potential "therapeutic window" in which targeting OCA-B leaves T cell development and primary immune responses intact. In contrast to primary stimulation, resting and re-stimulating previously activated Oct1- or OCA-B-deficient cells results in gene expression defects of 100-fold or more. Further, both OCA-B and Oct1 are required for CD4 memory formation and memory recall responses in vivo. Such repeated antigen exposures are also associated with autoimmunity. Here, we test the effects of T cell-specific deletion of Oct1 and OCA-B in mouse models of multiple sclerosis (MS) and type-1 diabetes (T1D). Oct1 deletion completely protects mice using an experimental autoimmune encephalitis mouse model of MS driven by self-antigen. In contrast, infecting the same mice with the neurotropic JHM strain of Mouse Hepatitis Virus (JHMV) results in similar viral pathology, immune responses and clinical scores, and only a slight delay in viral clearance. We developed a new conditional Ocab (Pou2af1) allele, backcrossed to the NOD strain background, and show that T cell specific deletion completely protects mice from T1D onset. This protection was associated with reduced infiltrating CD4+ and CD8+ T cells and macrophages, and reduced expression of proinflammatory cytokines. In contrast, the draining pancreatic lymph nodes in the same mice showed no difference in T cell numbers. Finally, we developed membrane-penetrating peptide inhibitors of the interaction between OCA-B and its downstream target, Jmjd1a. Relative to control peptides, these reduced glucose levels in newly diabetic NOD mice, while reducing T cell infiltrate and proinflammatory cytokine expression in the pancreata. Together, the results indicate that Oct1 and OCA-B are potent regulators of autoimmune responses, and promising candidates for pharmacological inhibition.

Presenter: Tellez Freitas, Claudia M.

CD5 co-receptor plays a role in T cell metabolism

Claudia M. Tellez Freitas, Tyler D. Cox, Anne Dunn, and K. Scott Weber

Department of Microbiology & Molecular Biology, Brigham Young University

During activation, T cells undergo metabolic reprogramming, which helps determine their distinct functional fates. CD5 is a co-receptor found on T cells and plays a significant role in regulating T cell thymic development, signaling, and cytokine production. Although CD5 is best known as a regulatory coreceptor during selection in T cell development, it has been reported to play a regulatory role similar to PD-1 and CTLA-4 upon T cell activation. We have found that CD5 levels influence calcium mobilization and T cell activation. Differential calcium mobilization, calcineurin function, and NFAT are known to affect glycolysis and mitochondrial respiration. Previous studies have shown that CD5 knockout mice (CD5KO) have increased T cell activation, leading to elevated levels of cytokine production and T cell proliferation. These functional changes suggest that CD5 may be affecting metabolic reprogramming. We hypothesized that CD5 deficient T cells have different bioenergetic demands that affect metabolic pathways key to T cell activation and function. We evaluated the effects of the CD5 co-receptor on metabolism by measuring the metabolic profiles of CD5KO and wild type T cells. Our preliminary data suggests that CD5KO T cells have higher mitochondrial respiration than wild type T cells and we are examining the mitochondrial mass in CD5KO naive T cells. Thus, CD5 may play an important role in metabolic programing in T cells and could potentially be useful used in modulating the T cell response in the tumor microenvironment.

Presenter: Thelin, Martin A

Enrichment of diabetogenic T cells in vivo using implantable scaffolds

Martin A Thelin, Stephan Kissler, Frederic Vigneault, Alexander L. Watters, Des White, Sandeep T. Koshy, Sarah A. Vermillion, David J. Mooney, Omar A. Ali, Thomas Serwold**

Joslin Diabetes Center at Harvard Medical School, Wyss Institute for Biologically Inspired Engineering at Harvard University, and University of California, San Francisco

Type 1 diabetes (T1D) is the most common chronic autoimmune disease in children and its prevalence is increasing globally. When a child is diagnosed with T1D, the immune system has already eradicated a great number of the insulin-producing cells and subsequently, a life-long insulin replacement therapy is required. T cells are known to play an essential role in the development of T1D both in humans and non-obese diabetic (NOD) mice. A major roadblock in the study of T1D is that the T cells that promote T1D, while abundant in the pancreas, are exceedingly rare in the blood. Currently, there is no efficient way of capturing T cells during the development of T1D without removing the pancreas. In order to enable the study of rare β-cell specific T cells, we developed an implantable scaffold to enrich for diabetogenic T cells. To determine whether scaffolds recruit populations of T cells that are similar to the T cell populations found in the pancreas, we loaded the scaffolds with beta cell lysates and implanted them subcutaneously onto the backs NOD mice. After 2 weeks, the scaffolds were harvested and autoimmune T cells were analyzed. We found that antigen-specific T cells become enriched within scaffolds containing their cognate antigens. These T cells induced diabetes after adoptive transfer, indicating their pathogenicity. Furthermore, T-cell receptor (TCR) sequencing identified many expanded TCRs within the β-cell scaffolds that were also expanded within the pancreata of NOD mice. These data demonstrate the utility of biomaterial scaffolds loaded with disease-specific antigens to identify and study rare, therapeutically important T cells.

Presenter: Tomala, Jakub

Design and production of IL-2-antibody single-chain fusions for cancer immunotherapy

Jakub Tomala[*+], *Seth Ludwig*[+], *Huilin Yang*[+], *Michael Leff*[+], *Marek Kovar**, *Jamie Spangler*[+]

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Interleukin-2 (IL-2) is a multifunctional cytokine that plays a critical role in immune cell differentiation, growth, and activation. Due to its ability to potently stimulate immune effector cells (i.e. CD8+ T cells and natural killer [NK] cells), IL-2 has been used extensively in cancer immunotherapy. Unfortunately, its concurrent promotion of regulatory T cells (Tregs) and harmful off-target effects have limited its clinical efficacy. Moreover, the serum half-life of IL-2 is vanishingly short (< 8 min), further complicating its use as a drug. Boyman and colleagues demonstrated that complexes of mouse IL-2 (mIL-2) with the anti-mIL-2 monoclonal antibody (mAb) S4B6 dramatically enhanced proliferation of immune effector cells compared to free IL-2 or S4B6 administration (1), presenting the exciting possibility of using antibody-cytokine complexes as therapeutic tools. However, therapeutic administration of these complexes is limited by the need for optimization of the cytokine:antibody dosing ratio and concerns of complex dissociation in vivo. To overcome these limitations, we designed, engineered and produced protein chimera consisting of mIL-2 linked to light chain of anti-IL-2 mAb S4B6 through a flexible oligopeptide spacer (2). In addition to improving upon the stability and therapeutic potential of cytokine-antibody complexes, our single-chain fusions have the potential to exhibit superior biological activity by circumventing cytokine dissociation. We showed the single-chain fusions we produced contain both IL-2 and S4B6 mAb in a single molecule and IL-2 interacts with binding site of S4B6 mAb in cis (1). We are now building on this powerful result to develop other translationally relevant cytokine-antibody fusions that could be used to address a wide range of disease challenges. 1.Boyman O, Kovar M, Rubinstein MP, Surh CD, Sprent J. Selective stimulation of T cell subsets with antibody-cytokine immune complexes.

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Presenter: Voss, James

Reprogramming the antigen specificity of B cells using genome-editing technologies

James Voss, James E Voss, Alicia Gonzalez-Martin, Raiees Andrabi, Roberta P Fuller, Ben Murrell, Laura E McCoy, Katelyn Porter, Deli Huang, Wenjuan Li, Devin Sok, Khoa Le, Bryan Briney, Morgan Chateau, Geoffrey Rogers, Lars Hangartner, Ann J Feeney, David Nemazee, Paula Cannon, Dennis Burton

The Scripps Research Institute

We have developed a method to introduce novel paratopes into the human antibody repertoire by modifying the immunoglobulin genes of mature B cells directly using genome editing technologies. We used CRISPR-Cas9 in a homology directed repair strategy, to replace the heavy chain (HC) variable region in B cell lines with that from an HIV broadly neutralizing antibody, PG9. Our strategy is designed to function in cells that have undergone VDJ recombination using any combination of variable (V), diversity (D) and joining (J) genes. The modified locus expresses PG9 HC which pairs with native light chains resulting in the cell surface expression of HIV specific B cell receptors (BCRs). Endogenous activation-induced cytidine deaminase (AID) in engineered cells allowed for Ig class switching and generated BCR variants with improved anti-HIV neutralizing activity. Thus, BCRs engineered in this way retain the genetic flexibility normally required for affinity maturation during adaptive immune responses.

Presenter: Webb, Lauren M.

Notch signaling in basophils controls a basophil-Th2 cell interplay during helminth-induced type 2 inflammation

Lauren M. Webb, Oyebola O. Oyesola, Simon P. Frueh, Elena Kamynina, Katherine M. Still, Ravi Patel, Seth A. Peng, Rebecca L. Cubitt, Andrew W. Grimson, Jennifer K. Grenier, Tajie H. Harris, Charles G. Danko, and Elia D. Tait Wojno

Cornell University

Type 2 inflammation is characterized by production of the cytokines IL-4, -5, -9, and -13 and promotes clearance of gastrointestinal helminth parasites, which infect over 2 billion people worldwide. Basophils are innate immune cells that support host-protective type 2 inflammation during helminth infection with Trichuris muris. However, the mechanisms that control basophil function and gene expression in this context remain unclear. We show that during T. muris infection, basophils localized to the intestine where they upregulated Notch receptor expression, rendering them sensitive to Notch signaling that can rapidly regulate gene expression programs. In vitro, Notch inhibition limited basophil IL-4 and IL-6 production in response to cytokine stimulation. Transcriptional profiling of Notch signaling-deficient basophils revealed that Notch directs basophil gene expression during helminth infection. Mice lacking basophil-intrinsic functional Notch signaling had impaired worm clearance, decreased intestinal type 2 inflammation, and altered basophil localization in the intestine, associated with decreased CD4+ Th2 responses following infection. These findings demonstrate that Notch regulates anti-helminth basophil gene expression and effector function, allowing these cells to support Th2 responses during type 2 inflammation.

Presenter: Wheibe, Elias

Evaluation of Hibiscone C derivatives in the Inhibition of Phosphatidylinositol-3-Kinase in T cells

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The phosphatidylinositol-3-kinase (PI3K) pathway is of significant interest due to its ability to regulate cell proliferation, growth, and migration in numerous contexts including in T cells. Furthermore, gain-of-function mutations in PI3K can lead to an upregulation of the pathway, resulting in tumorigenesis, autoimmunity, and leukemia. Specific furanosesquiterpenoids, such as wortmannin, have been known to inhibit the PI3K pathway in T cells. However, wortmannin has unfavorable characteristics as a chemotherapeutic or immunomodulatory drug due to its insolubility in neutral buffers and high toxicity in vivo. Hibiscone C, another furanosesquiterpenoid, lacks many of the functional groups as wortmannin, but contains the critical diacyl furan ring that is vital for interacting with the ATP binding pocket of PI3K . Via analysis of phosphorylation of the downstream effector molecule Akt in activated T cells, we demonstrate that Hibiscone C also can irreversibly inhibit PI3K activity, however not as effectively as wortmannin. These results led us to test other derivatives of Hibiscone C, to see if modifications to the molecules structure would affect potency of the molecule. Reduction of either of the carbonyl groups of the furan ring show reduced inhibitory activity compared to Hibiscone C. Reduction of both carbonyls also shows reduced potency but in addition abolishes the irreversibility of the inhibition. Modifications that increase electrophilicity show similar inhibitory activity compared to Hibiscone C. These data suggest that while these carbonyls are important for maximal binding to PI3K, they are not absolutely required to achieve inhibition, while the addition of electron withdrawing groups could optimize PI3K inhibition. These findings provide avenues for further manipulations of Hibiscone C functional groups to maximize efficacy of the molecule.

Presenter: Wijeyesinghe, Sathi

Quantifying the tissue-resident immune system

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Parabiosis studies have demonstrated that nonlymphoid tissues (NLTs) are primarily surveyed by tissue-resident memory CD8 T cells (TRM), independently of circulating memory T cells. (TRM) are established in nonlymphoid tissues following infection and mediate protective immunity during pathogen re-exposures. However, the protective utility of TRM is dependent on their longevity within nonlymphoid tissues. To test this critical aspect of TRM biology, we sought to rigorously enumerate TRM at multiple timepoints following a murine infection. Consistent with previous findings, we observed that memory T cells within circulation exhibited no decay over 450 days following infection. In nonlymphoid tissues, we observed tissue-specific changes in the frequency of mucosal TRM over time, which could be approximated by exponential decay models. However, tissue residence is not unique to T cells. Parabiotic surgery of congenically distinct mice can be used to identify any immune population that exists in NLTs independently of replenishment from circulation. We used parabiosis to distinguish tissue-resident CD45+ leukocytes from bloodborne leukocytes that entered nonlymphoid tissues over the duration of 30-day parabiosis. In all nonlymphoid tissues surveyed, over 50% of immune cells were tissue-resident over the course of 30-day parabiosis. Moreover, in specific mucosal tissues including the intestinal tract and female reproductive tract, 70-90% of the immune system was tissue-resident. These studies highlight the discordance in the immune system between vasculature, lymphoid tissues and nonlymphoid tissues. TRM exhibit distinct population dynamics within nonlymphoid tissues, not reflected by peripheral blood sampling, and the majority of the immune system is self-renewing within nonlymphoid tissues.

Presenter: Yamashiro, Livia

STING controls HSV infection in a type I IFN-independent manner

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Stimulator of interferon genes (STING) is an adaptor protein important for recognition of DNA from various pathogens. The main downstream event following STING activation is type I IFN signaling, with distinct consequences varying on bacterial or viral infections. STING can also orchestrate other downstream signaling events such as NF-kB activation and the initiation of autophagy. However, little is known of how these pathways are regulated, or whether these ancestral functions are still an important aspect of STING signaling in innate immunity against pathogens. Using mice that carry specific mutations on STING we were able to differentiate the downstream events of STING activation in the context of HSV infection. We show that the innate immune response to HSV is STING-dependent but, surprisingly, type I IFN-independent. Instead, we found that STING-dependent autophagy results in increased CD8+ T cells, suggesting augmented antigen presentation and viral clearance. Therefore, characterization of the distinct arms downstream of STING activation improves the understanding of their contribution during infections.

Presenter: Zebertavage, Lauren

Radiation Therapy Permits CD8+ T Cell-Mediated Control of Tumors through Tumor Cell Phenotype Alterations

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Background: Radiation therapy is traditionally used to induce catastrophic DNA damage leading to the death of rapidly-proliferating cancer cells and reduction of local tumor burden. While this basic premise remains true, it is now recognized that radiotherapy (RT) can trigger potent and effective anti-tumor immunity, primarily mediated by CD8+ T cells (CD8s), although the mechanisms by which this happens are unclear. Previously, we demonstrated that RT required pre-existing CD8 immunity to tumor antigens to drive clearance, and only moderately increased tumor reactive CD8s in circulation, indicating that RT functions poorly as an *in situ* vaccination. Interestingly, we observed that cytotoxic T cells were unable to control tumor cell growth *in vitro* unless the targets had been pretreated with RT or IFNγ. Hence, instead of directly altering T cell effector function, we questioned whether RT might render tumor cells more susceptible to CD8-mediated cytotoxicity via direct changes in tumor cells.

Hypothesis: We hypothesized that RT altered tumor cell phenotypes to increase susceptibility to CD8-mediated tumor regression *in vivo*.

Methods: For these studies, we used a murine model of pancreatic adenocarcinoma expressing the model antigen SIYRYYGL (SIY) and purified for high expression of the antigen (Panc02SIY100). Treatment of Panc02SIY100 with single-dose radiation (12 Gy) with αPD-L1 blockade results in tumor regression and clearance greater than 50% of mice. CD8+ T cells used for adoptive cell transfer (ACT) and *in vitro* assays were derived from 2C (SIY-specific) or OT-I (SIINFEKL-specific) transgenic TCR splenocytes activated *in vitro* with αCD3, αCD28 and IL-2 and purified with magnetic beads prior to administration. Irradiation was performed *in vitro* by timed exposure to radioactive Cs-137 and *in vivo* using the Xstrahl Small Animal Radiation Research Platform (SARRP). To generate MHC-I^{hi} tumor cell lines, cells were transfected with a plasmid harboring the MHC Class I TransActivator (CITA, or NLRC5) under the constitutive EF-1α promoter and purified for cell-surface MHC-I by FACS and clonal expansion.

Results: Using a $Rag1^{-/-}$ dual flank tumor model with adoptive transfer of CD8+ T cells, we found that RT-mediated tumor suppression was dependent on the presence of SIY-specific CD8s (d29 vs OT-I, p=0.023), while these cells had no effect on the size of unirradiated tumors in the same mouse (d29 vs OT-I, p=0.1938). We compared 2C vs OT-I infiltration into irradiated vs untreated tumors and found that RT did not draw more SIY-specific CD8s into the tumor (d7, per mg tumor, p=0.0637) and did not retain more 2C vs OT-I CD8s intratumorally (p=0.5435). We demonstrated *in vitro* that IFNγ (p=0.0023) and RT (10 Gy, p=0.0005, 20 Gy, p<0.0001) increased cell-surface expression of MHC-I by flow cytometry. Significantly, we observed that Panc02SIY100 was poorly controlled by 2C cells *in vitro* (p=0.6057) unless pretreated by IFNγ (p=0.0426) or constitutively expressing high levels of MHC-I (p=0.0074).

Conclusion: Taken together, these data support the conclusion that, in this model, tumor cell phenotype modifications driven by RT are essential to CD8-mediated tumor regression and support MHC-I upregulation as a potential mechanism driving this improved anti-tumor response by pre-existing tumor-specific T cells.

Presenter: Zhang, Ai-Hong

Suppression of FVIII-specific memory B cells by regulatory T cells expressing FVIII domains that interact with B-cell receptors

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Up to 1/3 of hemophilia A patients who receive FVIII replacement therapy develop neutralizing anti-FVIII antibodies, which renders the life-saving replacement therapy ineffective. We have recently shown that human regulatory T cells (Tregs) expressing a FVIII-domain as a chimeric B-cell antibody receptor (BAR) prevented anti-FVIII antibody formation in hemophilic mice by directly suppressing B cells (Zhang AH, J Immunol. 201:1434-1441, 2018). However, it is not known whether FVIII-specific memory B cells could be targeted by the FVIII BAR Tregs. To test this in vitro, we used plasmablast-depleted splenocytes from FVIII immunized hemophilia mice to serve as the source of FVIII-specific memory B cells. Co-culture of CD138- splenocytes with FVIII antigen in the presence of FVIII A2-domain BAR human Tregs completely blocked the development of FVIII-specific antibody forming cells (ASC). In addition, FVIII A2-BAR human Tregs efficiently suppressed FVIII-specific ASC formation even in the presence of anti-FVIII antibodies in vitro. In conclusion, we demonstrated in vitro that FVIII BAR Tregs suppress FVIII-specific memory B cells activity. The efficacy of FVIII BAR Tregs on FVIII-specific memory B cells will be further tested in vivo by adoptive transfer of FVIII-specific memory B cells in syngeneic hosts.

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