Presenter: Reich-Zeliger, Shlomit

Multiparameter mass cytometry of T cell thymic development and selection

Shlomit Reich-Zeliger, Avishai Gavish, Tomer M Salam, Arik Ryvkin, Nir Friedman

Weizmann Institute of Science

T cell development and selection in the thymus play key roles in shaping the adaptive immune system and maintaining self-tolerance. Using mass-cytometry (CyTOF) with 42 channels simultaneously measured, we generated a single-cell profile of the mouse thymus. CyTOF data provided information on T cell development and its regulation based on a panel of T-cell surface markers and transcription factors, as well as data on T cell activation using a Nur77 reporter, and on cell cycle stage and proliferation by injection of IDU-I127, a CyTOF compatible DNA synthesis marker. Using the newly developed Wishbone algorithm [1], we recovered the known stages in T cell development (DN, DP, CD4+ and CD8+) with high accuracy and developmental resolution. Of note, our approach allowed for precise construction of a branched developmental trajectory from one snapshot of un-sorted cells and without any genetic manipulation or other perturbation. Information on cell proliferation and cell cycle together with evaluation of cell density along the trajectory allows for developing a new algorithm that estimates the timing of developmental stages. Correlations between levels of TCR activation and expression of key transcription factors, which can be computed from single cell CyTOF data, provides insights on the regulation of CD4 vs. CD8 differentiation. Together, our findings provide a new and detailed description of the chronology of thymic T cell development and its regulation. [1] Setty M, Tadmor MD, Reich-Zeliger S, Angel O, Salame TM, Kathail P, Choi K, Bendall S, Friedman N, Pe'er D, "Wishbone identifies bifurcating developmental trajectories from single-cell data", Nat Biotechnol. 34, 637-45 (2016).

Presenter: Aarreberg, Lauren

Interleukin-1[b] induces innate antiviral gene expression via activation of IRF3 and STAT signaling

Lauren Aarreberg, Courtney Wilkins, Hilario Ramos, Adriana Forero, Justin Roby, Michael Gale, Jr.

University of Washington

Defense against microbial infection requires rapid detection of the pathogen and an appropriate cellular response. This process is driven by the recognition of pathogen- or danger-associated molecular patterns (PAMPs or DAMPs) by pattern recognition receptors to initiate intracellular signaling pathways that culminate in the expression of host defense molecules. These may include cytokines like interferon (IFN) and/or interleukin-1β (IL-1β). IFN acts to induce hundreds of genes that promote an antiviral state in infected and bystander cells. IL-1β, a product of inflammasome activation, is a potent inducer of NF-κB-responsive genes, whose products mediate inflammatory and immunomodulatory actions. IL-1 receptor (IL-1R) signaling also induces activation of the kinase TBK1, though a physiological role for this kinase in IL-1R signaling has not been elucidated. Here we report that exogenous IL-1β induces TBK1-mediated IRF3 activation and IRF3-dependent innate immune response genes in mouse myeloid cells, and in human myeloid and epithelial cells. IL-1β-induced IRF3 activation is dependent upon the essential DNA sensing pathway adaptor, stimulator of interferon genes (STING). We provide evidence that IL-1β-mediated STING activation likely occurs through the recognition of mitochondrial DNA in the cytosol. We demonstrate that exogenous IL-1β potentiates PAMP-induced IFN production and STAT signaling to amplify the innate immune response. Additionally, we show that IL-1R is required for maximal IRF3-mediated IFN production and immune activation in response to various PAMPs and RNA virus infection. These studies identify a new role for IL-1R signaling in the onset and/or enhancement of the actions of IFN and IFN-stimulated genes, with exciting new implications for the role of STING in integrating antiviral and inflammatory cues for host defense at barrier surfaces.

Presenter: abdelfattah, ahmed

Effect of selected osteopathic lymphatic techniques on immune system in healthy subjects

Ahmed Abdelfattah, Neveen Abdel Raoof, Samy Nasef , Rania ali , Rania Swailm

Faculty of Physical Therapy - Cairo University

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

Presenter: Acharya, Mridu

Alpha(v) integrins and autophagy proteins regulate germinal center B cell responses

Mridu Acharya, Fiona Raso, Lynda M. Stuart, Shaun Jackson, David J. Rawlings and Adam Lacy-Hulbert

Benaroya Research Institute

Signaling through Toll-like receptors (TLRs) can significantly impact the outcome of B cell activation by various types of antigens. TLR ligands associated with self-antigens can drive increased self-reactive B cell responses in autoimmunity, while TLR ligands present in pathogenic products or vaccines can also enhance protective antibody responses. Our recent work has identified a novel mechanism by which alpha (v) integrins, a family of adhesion molecules and the autophagy proteins limit excessive B cell TLR signaling. Specifically, we have found that alpha(v)beta3 integrin regulates B cell TLR signaling by directing maturation of TLR containing endosomes, through activation of components of the autophagy pathway. B cells lacking either alpha(v) integrins or autophagy components show enhanced TLR signaling and increased proliferative responses to TLR ligands. Mice with disruption in this pathway develop increased autoantibodies with age and develop accelerated autoimmunity in murine lupus models. Therefore, we propose that alpha(v)-mediated regulation of TLR signaling exists to limit excessive B cell responses to self-antigens. In addition, in current studies we find that alpha(v) integrins and autophagy proteins also regulate germinal center B cell responses to TLR ligands containing foreign antigens. Mice in which alpha(v) is specifically deleted from B cells, mount stronger antibody responses when immunized with exogenous antigens containing TLR-ligand adjuvants as well as virus like particles. Immune responses to these antigens are characterized by increased expansion of germinal center cells, increased somatic hyper-mutation and production of higher levels of high-affinity class-switched antibodies. Furthermore, loss of alpha(v) also leads to increased germinal center dependent long-lived antibody responses to influenza virus. These data indicate that the release of a regulatory mechanism previously associated with autoimmunity could be beneficial in enhancing protective antibody responses to viral antigens.

Presenter: Akilesh, Holly

Dysregulated TLR7 signaling drives anemia via development of unique hemophagocytes

Holly Akilesh, Matthew Buechler, Jeffrey Duggan, William Hahn, Xizhang Sun, Griffin Gessay, Elizabeth Whalen, Michael Mason, Keith Elkon, Adam Lacy-Hulbert, Marion Pepper, and Jessica Hamerman

Benaroya Research Institute

Cytopenias are an important clinical problem associated with acute inflammatory disease and infection. We show that specialized phagocytes that internalize red blood cells develop in TLR7-driven inflammation and during mouse malaria infection, both associated with severe anemia. TLR7 signaling caused development of inflammatory hemophagocytic cells (iHPC) that resemble splenic red pulp macrophages (RPM), but are a distinct population derived from Ly6Chi monocytes independently of the RPM lineage-defining transcription factor Spi-C. These iHPC were responsible for anemia and thrombocytopenia in TLR7-overexpressing mice, which have a severe Macrophage Activation Syndrome (MAS)-like disease. Our findings uncover a novel mechanism by which TLR signaling specifies monocyte fate during inflammation, and identify a new population of phagocytes responsible for anemia associated with inflammation and infection.

Presenter: Anderson, Kristin
Engineering adoptive T cell therapy for efficacy in ovarian cancer
Kristin Anderson, Breanna M. Bates, Edison Y. Chiu, Christopher B. Morse, Nicolas Garcia, Philip D. Greenberg
Fred Hutchingson Cancer Research Center and University of Washington

Over 20,000 women are diagnosed with ovarian cancer annually, and more than 50% will die within 5 years. This rate has changed little in the last 20 years, highlighting the need for innovative therapies. One promising new strategy has the potential to control tumor growth without toxicity to healthy tissues, by employing immune T cells engineered to target proteins uniquely overexpressed in tumors. Recently, mesothelin (MSLN) has been identified as a valid antigen target in ovarian cancer; MSLN contributes to malignant and invasive phenotypes and has limited expression in healthy cells. In preclinical studies using patient-derived cell lines or the mouse ID8 ovarian tumor model, we found that T cells engineered to express a high-affinity MSLN-specific T cell receptor (TCR) can kill ovarian tumor cells in vitro. In vivo, adoptively transferred TCR-engineered T cells preferentially accumulate within disseminated ID8 tumors, delay tumor growth and prolong mouse survival, but our data also show the tumor microenvironment (TME) can limit engineered T cell persistence and function. Immunosuppressive cells, inhibitory ligands that reduce T cell function, and cell death-inducing ligands are abundant within ID8 tumors. Further, the ovarian cancer TME is a nutrient- and oxygen-deprived milieu, and adaptive metabolic responses by infiltrating T cells have protean effects on T cell function. Cellular and molecular analyses of human ovarian cancer specimens showed similar TME-mediated obstacles exist for human T cell therapy. Ongoing studies will be discussed that are exploring strategies to overcome elements common to the human and murine TME, including direct modulation of the environment and T cell engineering to promote T cell survival and function.

Failure to suppress CYLD-mediated cell death causes immune dysregulation.

Rosalind Ang

The Precision Immunology Institute at the Icahn School of Medicine at Mount Sinai

SHARPIN together with RNF31/HOIP and RBCK1/HOIL-1 form the linear ubiquitin chain assembly complex (LUBAC) E3 ligase that catalyzes M1-linked poly-ubiquitination. Mutations in RNF31/HOIP and RBCK/HOIL-1 in humans and Sharpin in mice lead to auto-inflammation and immunodeficiency but the mechanism underlying the immune dysregulation remains unclear. We now show that the phenotype of the Sharpin-/- mice is due to the failure to inactivate CYLD, the deubiquitinase that removes K63-linked poly-ubiquitin chains. The dermatitis, disrupted splenic architecture, and loss of Peyer's patches in the Sharpin-/- mice were fully reversed in Sharpin-/-Cyld-/- mice. In Sharpin-/- cells, there is impaired phosphorylation of CYLD, which physiologically inhibits its deubiquitinating activity. Without this suppressive modification, CYLD removes poly-ubiquitin from RIPK1 in response to TNF, favoring the association of RIPK1 with death-signaling molecules to initiate death in Sharpin-/- cells, which was reversed in Sharpin-/-Cyld-/- cells. The dermatitis in the Sharpin-/- mice was also ameliorated by the conditional deletion of Cyld using LysMcre indicating that CYLD-dependent death of myeloid cells is inflammatory. Our studies reveal that under physiological condition, TNF- and RIPK1-dependent cell death is suppressed by the linear ubiquitin-dependent phosphorylation and inactivation of CYLD. The Sharpin-/- phenotype illustrates the pathological consequences when CYLD inhibition fails.

Presenter: Apostol, April

Maternal immune perturbation expands developmentally-restricted HSCs and progenitors in offspring

April Apostol, Anna Beaudin

University of California Merced

During development, the immune system is formed in a

Presenter: Arnold, Isabelle C.

Eosinophils suppress Th1 responses and restrict bacterially induced gastrointestinal inflammation

Isabelle C. Arnold, Isabelle C. Arnold, Mariela Artola-Boran, Paulino Tallón de Lara, Andreas Kyburz, Christian Taube, Karen Ottemann, Maries van den Broek, Anne Müller

University of Zurich

ABSTRACT TEXT NOT AVAILABLE ONLINE

Presenter: Barrett, Holly

Identification and production of Corynebacterium pseudotuberculosis proteins for pigeon fever vaccines

Holly Barrett, Jose Campuzano, Karen Molinder, Roberta Pollock

Occidental College

Corynebacterium pseudotuberculosis is a gram positive, facultative anaerobe that causes the equine disease pigeon fever. There are three possible manifestations of the disease: external abscesses, ulcerative lymphangitis, and internal abscesses. Internal infections have a high mortality rate of 30-40%, but no successful vaccine currently exists. Our lab focuses on vaccine development by testing *C. pseudotuberculosis* proteins in a mouse model. We first focused on the production of recombinant *equi* phospholipase D (PLD), a major exotoxin released by *C. pseudotuberculosis* and mutated, enzymatically inactive PLD. Various parameters were tested throughout the purification of both recombinant proteins to determine optimal conditions that yield high concentration and high purity of wild-type and mutant *equi*PLD. Next, we tested the ability of these recombinant proteins to protect mice from *C. pseudotuberculosis* infection. Sera from mice immunized with recombinant wild-type PLD, mutant PLD, and another recombinant protein, trehalose corynomycolyl transferase C (TCTC), were analyzed. Some cross reactivity was observed in this analysis, which we hypothesize is due to the 6-histidine tag used to isolate all recombinant proteins. To resolve this, both mutant PLD and wild-type PLD were treated with EnterokinaseMaxâ ¢ to remove the 6-histidine tags. Finally, we used the MudPIT technique to identify additional immunogenic *C. pseudotuberculosis* proteins that can be tested as potential vaccine components. Antibodies from externally infected, internally infected, or non-infected horse sera were used to immunoprecipitate *C. pseudotuberculosis* proteins, which were then identified by MudPIT. Antibodies against *C. pseudotuberculosis* proteins present in horses with internal infections but not external infections could be developed into a diagnostic assay for internal abscesses.

Presenter: Barry, Kevin C.

Characterization of the molecular and cellular mechanisms controlling protective myeloid cells in cancer

Kevin C. Barry, Joy Hsu, Miranda L. Broz, Francisco J. Cueto, Mikhail Binnewies, Alexis J. Combes, Amanda E. Nelson, Kimberly Loo, Raj Kumar, Michael D. Rosenblum, Michael D. Alvarado, Denise Wolf, Dusan Bogunovic, Nina Bhardwaj, Adil Daud, Joshua L. Pollack, Bushra Samad, Saurabh Asthana, Vincent Chan, Matthew F. Krummel

University of California, San Francisco

ABSTRACT TEXT NOT AVAILABLE ONLINE

Presenter: Batista, Samantha

IL-1[a] facilitates the entry of immune cells into the brain during chronic Toxoplasma gondii infection

Samantha Batista, Carleigh O'Brien, Tajie Harris

University of Virginia

Toxoplasma gondii is an intracellular protozoan parasite that causes chronic infections in the brain. Chronic infection is asymptomatic in healthy hosts, but immunocompromised individuals experience severe, even fatal, disease in which quickly 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, making T. gondii an excellent model to study neuroinflammation and immune cell recruitment into the CNS. During chronic T. gondii infection, cell death occurs in focal areas of inflammation in the brain, suggesting the possibility of the release of inflammatory molecules from dying cells. The alarmin IL-1α is expressed in the brain, and its expression is upregulated by chronic T. gondii infection. IL-1α is expressed predominantly by brain-resident microglia and infiltrating macrophages, implicating these cell types as potential sources of this cytokine. Mice lacking IL-1α or its receptor (IL-1R1), but not IL-1β, have defects specifically in monocyte/macrophage infiltration into the brain during chronic infection, as well as increased parasite burden in the brain. IL-1α has been the subject of much less investigation than IL-1β, but may still rely on inflammatory cell death for its release. Indeed, this IL-1α-dependent immune response appears to be dependent on pyroptosis, as caspase-1/11-/- mice phenocopy IL-1R1-/- mice displaying decreased recruitment of myeloid cells into the brain and increased parasite burden. IL-1R1 is expressed largely on endothelial and other stromal cells in the brain vasculature, and IL-1R1-/- mice display decreased mRNA expression of the adhesion molecules ICAM-1 and VCAM-1 in the brain. Together, these data suggest that during chronic T. gondii infection, IL-1α released from a macrophage population in the brain through pyroptosis acts on the brain vasculature to modulate expression of adhesion molecules, facilitating the infiltration of monocytes to sites of infection.

Presenter: Binnewies, Mikhail

High Dimensional Analysis of Myeloid Cells Reveals Regulatory T Cell Control of Lymph Node Priming

Mikhail Binnewies, Adriana Mujal, Joshua Pollack, Matthew Krummel

UC San Francisco

CD4 T cells have been shown to play a critical role in supporting productive anti-tumor responses. Less clear is how CD4 T cell responses are initiated and organized in the tumor draining lymph node (tdLN). Here we utilize a combination of single-cell RNA sequencing and high dimensional flow cytometry to parse the full extent of heterogeneity present within the tdLN and functionally assess the ability of each antigen presenting cell to prime tumor-antigen specific naÏve CD4 T cells. We identify two distinct IRF4-dependent CD11b+ dendritic cell (DC) populations that are uniquely able to prime naÏve tumor-antigen-specific CD4 T cells in the tdLN. While the ability to initiate CD4 priming is intact, these CD11b+ DCs are functionally and spatially restrained by regulatory T cells (Treg), where upon Treg depletion, CD11b+ DCs display enhanced migration and improved function, resulting in superior CD4 T cell priming and CD4 Th1 differentiation. Data from human tumors confirms not only the presence of a human equivalent of these two CD11b+ DC populations (BDCA-1+ CD14-/+ DCs) but also a relationship between BDCA-1+ DCs and CD4 Th. These findings reveal the complexity of CD4 T cell activation in the tdLN and highlight potential therapeutic avenues in boosting CD4 T cell function in cancer.

Effect of normal microbial exposure on the immune response to airway allergens

Katharine Block, Koji Iijima, Sara E Hamilton Hart, David Masopust, Hirohito Kita, Stephen C Jameson

University of Minnesota

Allergy and asthma are chronic conditions that can result in acute, life-threatening episodes. Our understanding of what makes individuals susceptible to these pathological immune responses is incomplete. Improvements in sanitation, vaccination programs, and other changes to individuals' microbial exposure are hypothesized to contribute to the rise in atopy in recent decades. This "hygiene hypothesis" posits that an immune system that has not been properly trained through fighting off natural infections is at risk of responding improperly to innocuous allergens, resulting in allergic asthma. We set out to experimentally test how this microbial training of the immune system influences responses to airway allergens using a murine model of normal microbial exposure. Laboratory mice are generally housed under specific pathogen free (SPF) conditions and their immune systems have a phenotype similar to a neonatal human with limited microbial exposure. Recent work has demonstrated that physiological animal-to-animal transmission of natural mouse pathogens alters the immune system and results in "dirty" mice with a phenotype that more closely resembles adults that have experienced a higher degree of microbial exposure. We used SPF mice to model children that have not been exposed to many infections or microbial products and "dirty" mice to model children with increased microbial experience and that appear to be protected from the development of allergy and asthma. We found that normal microbial exposure reduced recruitment of eosinophils into the lungs upon airway allergen exposure. Bulk CD4+ T cells, Th2 cells and allergen-specific CD4+ T cells were also decreased in dirty mice compared to SPF mice after allergen exposure. Future work will test whether dirty mice are resistant to lung inflammation and pathology in response to airway allergens and will investigate the parameters of allergen-specific CD4+ T cells responding in the lungs. The results generated from these studies will increase our understanding of how microbial exposures influence susceptibility to asthma and allergy. By addressing these questions, potential mechanisms underlying the development of allergy will be revealed, which could lead to intervention strategies.

Presenter: Bogoslowski, Ania

Neutrophils Recruited through High Endothelial Venules of the Lymph Node via PNAd Intercept Disseminating Staphylococcus aureus

Ania Bogoslowski, Eugene Butcher, Paul Kubes
University of Calgary

There is growing evidence that the lymph node may function as not only an adaptive immune organ but also as a barrier to disseminating pathogens. However, the immune cells involved and the mechanisms by which this occurs has not been fully elucidated. We demonstrate that Staphylococcus aureus breach across skin leads to some migration of the pathogen to the draining lymph node but no further. Profound neutrophil recruitment to the lymph node begins within the first hour after infection and plateaus at 6 hours. How neutrophils reach the lymph node and their role upon arrival is only beginning to be understood. Using intravital multiphoton imaging and the LysM eGFP reporter mouse we have visualized the behavior of neutrophils in lymph node blood vessels and tissue. Almost no neutrophils enter via lymphatics. Neutrophils accumulate within lymph node High Endothelial Venules, and transmigrate into lymph node tissue. Peripheral Node Addressin (PNAd) together with its two ligands, L-selectin and platelet P-selectin, was absolutely critical for recruiting neutrophils via the HEV. Finally, blocking neutrophil recruitment to lymph nodes depleted of resident macrophages results in higher bacterial load in draining lymph node and liver, showing the important role of the lymph node neutrophils in barrier function. Neutrophils actively phagocytosed S. aureus and helped sterilize the lymph node and prevent dissemination to blood and other organs. This study establishes neutrophils as part of the innate defence system of the lymph node.

Presenter: Brian, Ben F.

Dynamic regulation of LynA and c-Cbl impacts innate immunity

Ben F. Brian, Whitney L. Swanson, Frances V. Sjaastad, Tanya S. Freedman

University of Minnesota

Innate immune cells, like macrophages and mast cells, must be able to recognize and rapidly generate a response to diverse pathogenic stimuli. However, these cells must also be able to rapidly extinguish signaling to prevent the development of hyperinflammation that can produce tissue damage. Elucidating how signals are regulated downstream of these pathogen-sensing receptors is crucial to limiting harmful inflammation. We have discovered that the Src-family kinase LynA in macrophages is an important regulator of the antimicrobial response through immunoreceptor tyrosine-based activation motif (ITAM)-coupled receptors. We have shown previously that LynA is rapidly poly-ubiquitinated and degraded upon activation in macrophages, which prevents strong downstream signaling and the production of an immune response. Here we show that the ubiquitin ligase c-Cbl is responsible for poly-ubiquitinating LynA, and is a key negative regulator of ITAM signaling in macrophages. While macrophages express high amounts of c-Cbl and efficiently degrade LynA, we have shown that mast cells primarily express the closely related family member Cbl-b which is unable to poly-ubiquitinate LynA to the same extent. Differential expression of Cbl family members results in different signaling outcomes in mast cells and macrophages. We have also previously shown that macrophages stimulated with inflammatory cytokines like IFN-? upregulate LynA to amplify signaling downstream of ITAM-coupled receptors. Here we show that while LynA is upregulated in inflammatory conditions, c-Cbl is downregulated. Conversely, in anti-inflammatory conditions c-Cbl is upregulated and LynA downregulated. This dynamic regulation suggests that by altering the expression of membrane-proximal signaling components, environmental cues direct macrophage responsiveness, priming them for microbial elimination under inflammatory conditions and limiting aberrant inflammatory signaling in response to non-pathogenic stimuli.

Peritoneal podoplanin-expressing cells control peritoneal macrophage identity and localization

Matthew B Buechler, Shannon J Turley

Genentech

Peritoneal macrophages are important for tissue repair and pathogen clearance. The transcription factor GATA6 restricts their localization and expression of this gene is regulated by environmental factors, such as retinoic acid. Yet, the accessory cells that metabolize retinol and program peritoneal macrophages have not been identified. Here, we show that GATA6 and other lineage-defining genes are imprinted by non-hematopoietic podoplanin (PDPN)-expressing cells in the omentum. Surprisingly, we reveal that PDPN+ mesothelial cells, which ensheath all peritoneal tissues, exhibit a high Wt1-Raldh2 index that imbues GATA6 expression in peritoneal macrophages to restrict their localization to the peritoneal space in the steady state. During the macrophage disappearance reaction, peritoneal macrophage re-localized to the omentum via CCR1 ligand release by omental PDPN+ cells. Collectively, these results demonstrate that PDPN+ cells regulate gene expression and localization of peritoneal macrophages under homeostasis and LPS-induced inflammation.

Presenter: Cai, En

Microvilli enable efficient T cell antigen search and ligand detection

En Cai, Kyle Marchuk, Peter Beemiller, Casey Beppler, Matthew G. Rubashkin, Valerie M. Weaver, Audrey Gérard ,Tsung-Li Liu, Bi-Chang Chen, Eric Betzig, Frederic Bartumeus, Matthew F. Krummel

University of California-San Francisco

During antigen detection, T cells survey the surface of antigen-presenting cells (APCs), which typically display mainly nonstimulatory peptide-loaded major histocompatibility complexes (pMHCs) mixed with more rare cognate antigen in a process involving close (nanometer-scale) membrane apposition. T cells must thus solve a classic trade-off between speed and sensitivity. It has long been supposed that microvilli on T cells act as sensory organs to enable search, but their strategy has been unknown. We used lattice light-sheet microscopy and quantum dot-enabled synaptic contact mapping microscopy to show how microvilli on the surface of T cells search opposing cells and surfaces before and during antigen recognition. We uncovered that microvilli on T cell surfaces dynamically survey the majority of opposing surfaces within one minute through anomalous diffusion. T cell receptor (TCR) recognition resulted in selective stabilization of receptor-occupied protrusions, which was independent of tyrosine kinase signaling and the actin cytoskeleton. We now reveal that TCRs on activated T cells are non-uniformly distributed on cell membrane: some TCRs were concentrated on microvilli, while other TCRs formed microclusters on flatter membrane patches. Most of microvilli are TCR-enriched, but a small population of microvilli do not contain appreciable densities of TCR. A second feature of recognition is revealed by examining the opposing surface. We found that during T cell-APC interaction, T cell microvilli project deep into 3D pockets formed by veil structures on the surface of dendritic cells (DC); and DC membrane also conformed to accommodate T cell microvilli, which increased the effective close-contact area between T cell and APC. Such scanning patterns enable T cells to efficiently scan more APC surface in given time. This work defines the efficient cellular search process against which ligand detection takes place in T cells.

Presenter: Campbell, Daniel

Identification of functionally unique CD4 T cells that are the circulating counterparts of epidermal resident memory T cells

Daniel Campbell, Thomas Duhen, Maria Klicznik, Samantha Motley, Peter Morawski, Barbara Hoellbacher, Iris Gratz

Benaroya Research Institute

As a barrier organ the skin contains specialized T cell populations that combat infection and also help maintain tissue homeostasis and promote wound repair. Circulating skin-tropic T cells can also be identified in the blood based on their expression of the cutaneous lymphocyte antigen (CLA), but the developmental and functional relationships between these circulating CLA+ T cells and tissue-resident T cells in the skin are not fully understood. Using 33-parameter CyTOF (Cytometry by time-of-flight) analysis we identified a novel population of skin-tropic CD4+ T cells in human blood that expresses the CD103 integrin and phenotypically and functionally resembles epidermal resident memory T cells (TRM) in the skin. RNA sequencing identifies a set of signature genes shared by circulating CD103+CLAhi T cells in the blood and epidermal TRM cells in the skin, and suggests that CD103+CLAhi T cells contribute to normal skin function and response to tissue damage. Finally, using humanized NSG mice carrying full-thickness human skin grafts we demonstrate that despite their identification as TRM, CD103+CLAhi T cells in the skin are capable of exiting the tissue and re-entering the circulation. Thus, CD103+CLAhi T cells in the blood represent a circulating T cell population of cutaneous TRM, and this provides novel opportunities for the study and therapeutic manipulation of TRM cells in the contexts of cutaneous infection, inflammation and tissue-repair.

Presenter: Catalan-Dibene, Jovani

Identification of Interleukin 40, a novel B cell-associated cytokine

Jovani Catalan-Dibene, Monica I. Vazquez, Van Phi Luu1, Sean-Paul Nuccio, Alborz Karimzadeh, Jenna Kastenshmidt, S. Armando Villalta, Irina Ushach, Egest J. Pone, Amanda M. Burkhardt, Paolo Casali, Manuela Raffatellu, Marcela Hernandez, Gina Heller, Peter A. Hevezi and Albert Zlotnik.

University of California, Irvine

We have identified a novel mouse and human cytokine. IL-40 is produced in the bone marrow, fetal liver and by activated B cells. Its sequence predicts a small (~27kDa) secreted protein unrelated to other cytokine gene families, that we have called Interleukin 40 (IL-40). IL40 is only present in mammalian genomes, suggesting a role in mammalian immune responses. Accordingly, IL-40 expression is induced in the mammary gland upon the onset of lactation, and an IL-40-/- mouse exhibits reduced levels of IgA in the serum, gut, feces, and milk. Furthermore, the IL-40-/- mouse has smaller and fewer Peyer's patches and IgA secreting cells. The gut microbiome of IL-40-/- mice also exhibits altered composition, reflecting the reduced levels of IgA in the gut. Pre-B cells are altered in the bone marrow of the IL-40-/- mouse. Taken together, these observations indicate that IL-40 represents a novel B cell associated cytokine, that plays an important role in B cell development and humoral immune responses. IL-40 is also expressed by human activated B cells and by several human B cell lymphomas. The latter observation suggests that it may play a role in the pathogenesis of these diseases.

High-throughput interrogation of the HSV-1 genome under type-I IFN pressure

Maxime Chapon, Jeffrey S Zhao, Genhong Cheng

UCLA

ABSTRACT TEXT NOT AVAILABLE ONLINE

Toxoplasma and host interaction: the injection alone changes Macrophage Polarization

Longfei Chen, David Christian, Anita Koshy, Christopher A. Hunter
University of Pennsylvania

The ability of IFN-gamma to activate macrophages is associated with an M1 phenotype that includes increased anti-microbial activity and is critical for the ability to inhibit replication of Toxoplasma gondii. Alternatively, when type I and III strains of T. gondii infect macrophages they engage an M2 transcriptional program and generate a population of cells that are typically characterized as having poor anti-microbial activity. Here, in vitro and in vivo studies using parasites that express Cre we show that a group of macrophages that have been solely injected but which lack the parasite express an M2 phenotype. Thus, injection of parasite derived rhoptry proteins alone has a significant impact on host cell function and raises new questions about the persistence of these M2 populations and their impact on virulence and the outcome of infection.

Presenter: Chiu, Honyin

A Novel Role for the 4E-BP/eIF4E Axis in B Cell Antibody Class Switching

Honyin Chiu, Leandra Jackson, Anni Mai, and David A. Fruman

University of California, Irvine

During an adaptive immune response, activated mature B cells give rise to antibody secreting plasma cells to fight infection. B cells undergo a process known as antibody class switch recombination (CSR) to produce different classes of antibodies with varying effector functions. The mammalian target of Rapamycin (mTOR) signaling pathway is activated during this process and B cells deficient in mTOR have impaired survival, proliferation and differentiation into antibody secreting cells. It has been shown that antibody class switch recombination requires B cell division, however it is unclear if there is another regulatory mechanism through mTOR that is independent of proliferation. We and others have found that the mTORC1 allosteric inhibitor rapamycin, at non-immunosuppressive concentrations in vivo, can significantly decrease the amounts of germinal center B cells while still maintaining IgM secreting B cells. We have also found that rapamycin, when added after a B cell has committed to divide, can suppress class switching and cap-dependent translation while preserving proliferation. Additionally, restricting Glutamine availability phenocopies low concentrations of Rapamycin, in vitro. Mechanistic investigation of the mTORC1 substrate 4E-BP, an inhibitor of eIF4E and cap-dependent translation, showed that blocking the 4E-BP/eIF4E axis can decrease antibody class switching independently of proliferation. We have found that the mechanism involves decreased translation of the Aicda mRNA encoding activation-induced cytidine deaminase (AID) protein. These results uncover a novel role for mTORC1 and the 4E-BP/eIF4E axis in AID protein levels and B cell antibody class switching, suggesting that cap-dependent translation regulates key steps in B cell differentiation. Further study of this pathway may provide mechanistic insight into antibody-mediated autoimmune diseases such as lupus and arthritis as well as the formation of protective antibody responses following vaccination.

Presenter: Christian, David

cDC1 are essential for coordinating innate and adaptive immune responses during Toxoplasma gondii vaccination

David Christian, Gretchen Harms Pritchard, Anita A. Koshy, Christopher A Hunter

University of Pennsylvania

Intraperitoneal immunization with a live attenuated strain of Toxoplasma gondii generates minimal inflammation but also a robust and protective type 1 T cell response. Cytokines essential for a type I immune response (IL-12 and IFN-γ) and the Batf3-dependent type I conventional dendritic cells (cDC1s) are required to induce a parasite-specific, protective CD8+ T cell response. This CD8+ T cell response requires the spatiotemporal orchestration of antigen, antigen presenting cells, and CD4+ T cell help in draining lymphoid organs. The omentum is a visceral adipose tissue present in the peritoneal cavity containing fat associated lymphoid cluster (FALCs) termed "milky spots" that serve as sites to support a rapid immune response in the peritoneum. Despite the ability of these milky spots to form organized structures capable of T and B cell priming, little is understood about how inflammatory cells from the peritoneum are recruited to these sites. Using Batf3 KO mice, cDC1s are shown here to be a required source of IL-12 immediately after CPS immunization. This IL-12 activates natural killer (NK) cells to produce IFN-γ that induces the migration of peritoneal cells containing parasite antigen to sites of T cell priming in omental milky spots. By combining a Cre-secreting, mCherry+ strain of CPS with Cre reporter mice, the role of infected cells was tracked in vivo. Immunofluorescence microscopy of these milky spots provided understanding of how the architecture of these structures enables efficient T cell priming. These data have important implications for the design of vaccine strategies capable of eliciting a protective cellular immune response, and lend insight into the role of cDC1s to initiate an adaptive immune response in atypical secondary lymphoid structures.

BCAP promotes TLR-induced IFN[a] production in pDC

Talyn Chu, Hayley Waterman, Griffin Gessay, and Jessica A. Hamerman
University of Washington

Systemic lupus erythematosus (SLE) severity is correlated with elevated serum levels of type I interferons (IFN), specifically IFNα. pDC are important in the pathogenesis and etiology of SLE due to their ability to produce large amounts of IFNα in response to endocytosed nucleic acids. TLR7 and TLR9-induced IRF7 translocation to the nucleus and subsequent IFNα production by pDC is dependent on phosphatidylinositol-3 kinase (PI3K), but how PI3K is activated and regulates this process remains undefined. We showed that the cytosolic signaling adapter B cell adaptor for PI3K (BCAP) links TLRs to PI3K activation in macrophages, thus we asked whether BCAP plays a role in pDC IFNα production and SLE pathogenesis. Here, we show BCAP promoted many aspects of TLR7-driven lupus-like disease including interferon-stimulated gene expression in the blood. Consistent with these findings, we found BCAP promoted TLR7 and TLR9-induced IFNα production in pDC. Strikingly, BCAP-/- mice produced significantly less serum IFNα after injection of TLR9 agonist than WT mice, consistent with a pDC IFNα defect. TLR-induced IFNα production in pDC involves dual signaling pathways that run in parallel. There is a TLR-independent pathway that is initiated by ligand recognition at the plasma membrane and involves Dock2-mediated activation of Rac1, required for the phosphorylation of IKKα, and a TLR-dependent pathway that occurs upon RNA or DNA recognition by endosomal TLR7 or 9 respectively and leads to MyD88 activation. The pathways converge upon the phosphorylation and activation of IKKα leading to the phosphorylation and nuclear translocation of the transcription factor IRF7 and IFNα gene transcription. BCAP regulated IFNα production independently of the TLR-MyD88 pathway and both BCAP and PI3K activation were required for CpG DNA-induced early actin remodeling, a readout of Rac1 activation, and IKKα phosphorylation in pDC. These data suggest BCAP and PI3K specifically regulate the TLR-parallel pathway in pDC. Overall, we show a novel role for BCAP in regulating IFNα production in pDC and lupus pathogenesis.

Presenter: Condotta, Stephanie A.

A novel role for cyclophilin D in CD8 T cell-mediated antiviral immunity

Stephanie A. Condotta, Jeffrey Downey, Stefanie F. Valbon, Ryan D. Pardy, Esther Tarrab, Alain Lamarre, Maziar Divangahi, Martin J. Richer

McGill University

CD8 T cell-mediated antiviral immunity is critical for the host defense against viruses. Mitochondria play an important role in antiviral immunity and activation of type I interferon (IFN-I) is critical for optimal antiviral CD8 T cell responses. Cyclophilin D (CYPD) is a mitochondrial matrix protein that modulates the mitochondrial permeability transition pore. The role CYPD plays in CD8 T cell antiviral immunity remains poorly defined. Herein, we demonstrate that CYPD is important in the initiation of the CD8 T cell antiviral immune response by modulating IFN-I production in a CD8 T cell extrinsic manner. Reduced IFN-I results in compromised CD8 T cell antiviral immunity and increased pathogen burden. Further, we show that reduction in STAT1 activation in CYPD deficient animals is associated with compromised CD8 T cell antiviral immunity. These results indicate that CYPD is required for optimal effector CD8 T cell responses to viral infection. Furthermore, this suggests that, under certain circumstances, inhibition of CYPD function could have detrimental effects impacting the host's ability to respond to viral infection.

Presenter: Cortez Vollmers, Apple

Characterization of long non-coding RNA GAPLINC in mouse macrophages

Apple Cortez Vollmers, Author Names separated by commas

UC Santa Cruz

The innate immune system is the first line of defense against microbial and common bacterial infections. The first cells to respond to infection are macrophages, which are differentiated from cells called monocytes. Activated macrophages, in response to infection, trigger an inflammatory response through toll-like receptors (TLRs) that initiate the release cytokines and chemokines. Dysregulation of the inflammatory response following activation can lead to detrimental effects in the host such as septic shock, auto-immune disease, and cancer. More recently, long non-coding RNAs (lncRNAs) have been implicated to have functional roles in immunity. LncRNAs comprise the largest group of RNA transcripts produced by the cell, are greater than 200 nucleotides in length, and lack protein-coding ability. RNA-seq data acquired from primary human monocytes and macrophages identified a lncRNA called gastric adenocarcinoma predictive long intergenic noncoding (GAPLINC) to be highly upregulated (>1000-fold) during the monocyte to macrophage differentiation process. We observed that siRNA knockdown of GAPLINC in primary cells activated inflammatory response genes. Alternatively, increased GAPLINC during differentiation showed a decrease in inflammatory gene expression levels, but upon TLR4 stimulation, became highly attenuated. Here, we identify a mouse homolog of GAPLINC. Our findings recapitulate preliminary results found in human, as well as show that we can effectively target GAPLINC using CRISPRi technology.

SUMO-2/3 prevents the activation of a spontaneous Type I Interferon response

Ty Crowl, Daniel Stetson

University of Washington

Detection of intracellular nucleic acids by innate immune sensors triggers the production of Type I Interferons (IFNs), a family of cytokines that activate an antiviral response. While IFNs are critical for host defense against viral infection, dysregulated production underlies multiple autoinflammatory diseases in human patients. We have found that disruption of any essential ligase of the sumoylation pathway results in a potent and spontaneous IFN response. Vertebrates possess three small ubiquitin-like modifiers (SUMOs) that can be conjugated onto target proteins and alter protein function in diverse, but still poorly characterized ways. We have discovered that regulation of IFN by sumoylation is redundantly mediated by both SUMO-2 and SUMO-3, but not SUMO-1, revealing a previously unknown and specific function of SUMO-2/3. Remarkably, this IFN response is not dependent on any known nucleic acid sensing pathway and does not require either of the canonical, IFN-inducing transcription factors IRF3 or IRF7. Taken together, these findings demonstrate that SUMO-2 and SUMO-3 are essential regulators of Type I IFN responses that prevent the activation of a non-canonical and potentially novel mechanism of IFN induction.

Presenter: Cusick [1,*], John

RELT stains prominently in B cell lymphomas and binds the hematopoietic transcription factor MDFIC

John Cusick [1,], George Talbott[1], Yasmeen Alhomsy[2], Pachai Moua[1], Cara Sumida[3], Nazila Hejazi[2], Aaron Jacobs[3]*

[1] California Northstate University, College of Pharmacy, Elk Grove, CA 95757 [2] California Northstate University, College of Medicine, Elk Grove, CA 95757 [3] University of Hawaii at Hilo, College of Pharmacy, Hilo, HI 96720 [*] Current address: California University of Science and Medicine, San Bernardino, CA 92408.

ABSTRACT TEXT NOT AVAILABLE ONLINE

Presenter: De Jesus Carrion, Steven

Regulation of Tumor Growth by TSLP During Colorectal Cancer

Steven De Jesus Carrion, Steven F. Ziegler

Benaroya Research Institute

Thymic stromal lymphopoietin (TSLP) is a cytokine involved in promoting tumor growth during breast and pancreatic cancer by promoting Th2 cell-mediated inflammation. Despite being essential to maintain Treg cell homeostasis in the mouse and human intestine, a role for TSLP in colorectal cancer has never been shown. To determine if TSLP affects tumor growth during colorectal cancer, we utilized a murine model of colitis-associated colorectal cancer. TSLP deficient mice had fewer tumors when compared to WT littermate controls and TSLP receptor (TSLPR) expression was significantly upregulated after development of cancer. Further, deletion of the TSLP signal on intestinal epithelial cells (IECs) led to a significant decrease in tumor growth. In vitro, we found that TSLP signal activates the JAK2/STAT5 pathway in a colon cancer cell line, and chemical blockade of STAT5 phosphorylation led to increased apoptotic cell death, suggesting that TSLP is required for tumor survival. TSLP was also able to interact with the immune system to promote Treg cell differentiation to help the tumor grow, and in the absence of TSLP there was higher frequency of activated CD4+ T cells. Overall, these data show a novel role for TSLP in controlling tumor progression during colorectal cancer and identify it as a potential target for immunotherapy intervention.

Investigating a role for the NAIP/NLRC4 inflammasome in T cell immunity

Katie Deets, Russell Vance

UC Berkeley

The work that has been done thus far to investigate the role of the NAIP/NLRC4 inflammasome in generating an adaptive immune response has been carried out in the context of bacterial infections, which introduce a variety of PAMPs and make isolating specific response pathways difficult. To address this problem, the Vance lab has developed a novel in vivo genetic system in which we can specifically induce expression of flagellin (Fla), a potent activator of NAIP/NLRC4, along with ovalbumin (Ova) as a single fusion protein (OvaFla) in the cytosol of cells under the control of a tamoxifen-inducible Cre recombinase. With this tool, we will address whether NAIP/NLRC4 is sufficient to generate an adaptive T cell response through the following questions: (1) Are antigens released by pyroptotic cells taken up and displayed by antigen presenting cells? (2) What type of adaptive T cell response is generated? (3) Is NAIP/NLRC4 activation sufficient to generate a successful memory T cell response?

Presenter: DeRogatis, Andrea M.

Living on the Edge: Evaluating the Constitutive Innate Immunity of Anna's Hummingbirds (Calypte anna)

Andrea M. DeRogatis, Kirk C. Klasing, Lisa A. Tell

University of California, Davis

Hummingbirds are indispensable pollinators in many ecosystems, making their conservation essential. As is the case with many species, hummingbirds are now faced with new challenges resulting from anthropogenic changes. Unfortunately, there is currently a very limited understanding of what factors may influence the success of hummingbird conservation including changing disease dynamics and new diseases. There has been little research done investigating the pathogenic threats that hummingbirds face. Hummingbirds are the smallest vertebrates and have the highest metabolic rates, indicating that their immunological defense may differ from more commonly studied taxa. The objective of this study is to gain a basic understanding of the constitutive innate immunity of hummingbirds. This was evaluated for Anna's hummingbirds (Calypte anna) by examining the in vitro microbicidal capacity of whole blood. Age and sex were found to have no effect on the microbicidal killing ability of whole blood when exposed to E. coli. When multiple bacterial species were tested, there was a significant difference in the killing ability based on species. This basic knowledge improves conservation efforts by providing important insight into how different diseases may impact hummingbird populations based on their innate immunity.

Presenter: Dolina, Joseph S.

Toll-like receptor 9 is required for the maintenance of CD25+FoxP3+CD4+ Treg cells during Listeria monocytogenes infection

Joseph S. Dolina, Joey Lee, Stephen P. Schoenberger

La Jolla Institute for Allergy and Immunology

It has long been appreciated, but not understood, that the CD8+ cytotoxic T lymphocyte (CTL) dependence on CD4+ T cell help (Th) is conditional; needed for some immunogens but not others. One explanation for this phenomenon envisions Th requirement as an intrinsic property of the pathogen itself rather than its introduction to the immune system. Here we show that dependence of the optimal CD8+ T cell response to Listeria monocytogenes (Lm) on CD4+ T cells is a function of the immunogen dose used for priming, with low dose Lm (LD; 50 or 10^3 CFU WT or ΔActA, respectively) inducing a primary antigen-specific CTL response profoundly dependent on CD4+ Th cells while that induced by high dose Lm (HD; $4x10^3$ or 10^6 CFU WT or ΔActA, respectively) is significantly inhibited by CD4+CD25+FoxP3+ regulatory T cells (Treg). The Th-independence of HD immunization is not overcome by additional antigen but instead involves the inflammatory response to more bacteria. Evaluation of various TLR pathways as the relevant sensing mechanism showed that HD immunization in the absence of TLR9/IL-12 signaling results in a simultaneous loss of CD25+FoxP3+CD4+ Treg cells and increase in conventional CD4+ Th cells and CTLs. Our data thus reveal that the CTL response to the same pathogen is determined by distinct roles for CD4+ T cells as helpers versus regulators based on immunogen dose and demonstrate a previously undescribed role for TLR9 in the regulation of CD4+ Th and Treg cells.

Presenter: Dunai, Cordelia

Maintenance of the unlicensed NK cell subpopulation in F1 MHC hybrid mice

Cordelia Dunai, Ethan G. Aguilar, Lam T. Khuat, William J. Murphy

UC Davis

The activation of NK cells is controlled by the integration of activating and inhibitory signals. NK cells can be divided into subsets termed licensed and unlicensed based on the expression of inhibitory receptors with varying affinities for MHC class I molecules. Our group has shown that licensing delineates helper and effector NK cell subsets during viral infection. Unlicensed NK cells localized to lymph nodes and produced proportionately higher levels of GM-CSF which correlated with maturation of dendritic cells, which in turn fostered antigen-specific T cells. This division of labor occurs in mice and humans, but is mediated by different families of receptors. We studied two different strains of mice (C57BL/6 and BALB/c), as well as their F1 hybrids. These two strains have different MHC haplotypes and the F1 offspring express both, leading to different dynamics of licensed and unlicensed NK cells. NK cells expressing Ly49C/I, Ly49G2, and Ly49A are licensed in F1 offspring as these inhibitory receptors bind MHC class I molecules H-2Kb, H-2Db and H-2Dd, respectively. We have observed that to compensate for the increased frequency of licensed NK cells, there is a compensatory increase in the frequency of Ly49C/I-Ly49G2-Ly49A- NK cells, indicating the maintenance of the unlicensed subpopulation in F1 hybrids. Understanding more about the subsets of NK cells has relevance to clinical applications of de novo generated or adoptively-transferred NK cells for the treatment of cancer.

Presenter: Dynes, Joseph

Intermittent Ca2+ signals mediated by Orai1 regulate basal T cell motility

Joseph Dynes, Tobias X. Dong, Shivashankar Othy, Milton L. Greenberg, Amit Jairaman, Chijioke Akunwafo, Sabrina Leverrier, Ying Yu, Ian Parker, and Michael D. Cahalan

Department of Physiology & Biophysics, University of California, Irvine

Seemingly random patterns of movement help T cells search for antigen in the lymph node. Because prominent Ca2+ signals accompany T cell activation and lead to cell arrest and dramatic changes in cell morphology, we hypothesized that more limited Ca2+ signaling might tune patterns of interstitial T cell motility in the absence of antigen. Blocking Orai1 Ca2+ channel activity in human T cells by expression of a dominant-negative Orai1-E106A construct led to migration with higher average velocities than controls, both in reconstituted mouse lymph nodes in vivo and in confined microchannels in vitro. In particular, we found that the increase in average cell velocity was not due to an increase in maximum cell velocity, but to a reduced frequency of cell pausing accompanied by increased directional persistence, resulting in longer and straighter paths. Isolated human T cells demonstrated Ca2+ transient-associated and Orai1-dependent pauses in vitro within confined microchannels devoid of cell-extrinsic factors. Furthermore, we used a novel ratiometric genetically encoded Ca2+ indicator, Salsa6f, along with T cells from CD4-Salsa6f transgenic mice, to show that intermittent Ca2+ signals coincide with reduced cell velocity. Treatment of CD4-Salsa6f mice with MHC class-I and -II blocking antibodies substantially reduced but did not eliminate the frequent T cell Ca2+ transients seen in lymph nodes. These experiments support the existence of an Orai1-dependent cell motility program that leads to pausing and turning of T cells moving within lymph nodes. Such Orai1-dependent pauses are triggered in at least two different ways: by self-peptide MHC complexes displayed on the surface of APCs and by a novel cell intrinsic mechanism within the T cells themselves. Together, these mechanisms generate motility patterns that underlie immune surveillance in the lymph node.

Presenter: Elisa, Cruz

The intestinal lamina propria is a unique niche for regulatory T cells

Cruz Elisa, Terri Laufer

University of Pennsylvania

Foxp3+ regulatory T cells (Tregs) are critical to maintain intestinal homeostasis. Like all CD4+ T cells, Tregs require TCR-MHCII interactions for their development in thymus and differentiation in periphery. Past studies suggest that the TCR repertoire of intestinal lamina propria (siLP) Tregs is skewed towards food and commensal bacteria antigens. However, by using a transgenic mouse model that lacks peripheral MHCII expression and peripheral Tregs induction (K14 mice), we have demonstrated that thymically generated Tregs could enter to the siLP of weanlings and proliferate independently of MHCII to fill and maintain the compartment. Newly-generated Tregs egress from thymus as central Tregs and acquire an effector phenotype after TCR signals in periphery. However, despite the lack of MHCII in the siLP, K14 Tregs are activated and phenotypically similar to effector Tregs in WT siLP. Interestingly, transcriptional profiling reveals that many components of a TCR-dependent expression module are present in both K14 and WT siLP Tregs. Furthermore, differently to splenic Tregs, K14 siLP Tregs highly express Nur77 and IRF4, often regulated through TCR signaling. Tregs from K14 siLP localize mainly at isolated lymphoid follicles, where they are in proximity to B cells and dendritic cells; suggesting that siLP Tregs may still receive MHCII-independent signals from APCs, perhaps via costimulation molecules and/or paracrine cytokine stimulation. Indeed a short-term costimulation blockade with CTLA4-Ig reduces Nur77 expression on K14 siLP Tregs. Taken together, these results suggest that the intestinal microenvironment uniquely maintains the differentiation and homeostasis of effector Tregs independent of MHCII-TCR interactions.

Presenter: Feigelson, Sara

ICAMs are not obligatory for functional immune synapses between naÏve CD4 T cells and lymph node DCs

Sara Feigelson, Adam Solomon, Adi Biram, Miki Hatzav, Moria Lichtenstein, Ofer Regev, Stav Kozlovski, Diana Varol, Caterina Curato, Dena Leshkowitz, Steffen Jung, Ziv Shulman, Ronen Alon

Weizmann Institute of Science

Protective immune responses depend on the formation of immune synapses between T cells and antigen presenting cells (APCs). The two main LFA-1 ligands, ICAM-1 and ICAM-2, are co-expressed on many cell types including APCs and blood vessels. Although these molecules were suggested to be key players in immune synapses studied in vitro, their contribution to helper T cell priming in vivo is unclear. We used transgenic mice and intravital imaging to examine the role of DC ICAM-1 and ICAM-2 in naÏve CD4 T cell priming and differentiation in skin-draining lymph nodes. Surprisingly, ICAM deficiency on endogenous CD40-stimulated lymph node DCs did not impair their ability to arrest and prime CD4 lymphocyte activation and differentiation into Th1 and Tfh effectors. Thus, functional helper T cell synapses with antigen presenting DC and subsequent proliferation and early differentiation into T effectors generated during vaccination do not require LFA-1-mediated T cell adhesiveness to DC ICAMs. This is the first demonstration that TCR signals can promote functional immune synapses between naïve T cells and antigen presenting DCs via integrin-independent adhesions driving normal proliferation and differentiation inside vaccinated lymph nodes.

Presenter: Ganeshan, Kirthana

TLR4 activation triggers energy conservation programs in mice

Kirthana Ganeshan, Kevin Man, Yoshitaka Sogawa, Xiaojin Cui, Ajay Chawla

UCSF

Organisms acquire and allocate energy to support programs of growth, reproduction and tissue maintenance. Activation of immunity is a high-cost stress response that can force the organism to reallocate available energy to support programs that improve host fitness. Indeed, the activation of immunity can often pose a significant metabolic challenge for the host, and as such; it is likely that immunity may compete with host maintenance programs for energy. However, the nature of these physiologic trade-offs and their significance remain poorly understood. To investigate this question, we activated immunity in mice that had a competing need for energy to maintain homeothermy (mice housed under sub-thermoneutral conditions ($22°C$)). Using LPS-activated immunity as a model system, we determined that mice housed at $22°C$ choose to lower metabolic rate and abandon homeothermy in an effort to conserve energy as compared to thermoneutral mice (housed at $30°C$). The activation of this energy conservation program is dependent on hematopoietic sensing of LPS. Metabolomic analysis of plasma from the entry and exit phases of this programed hypothermia revealed that entry into hypothermia is supported by the catabolism of amino acids, while exit is fueled by fatty acids and the activation of UCP1-dependent thermogenesis. The decision to engage in such an energy-conserving program ultimately enhances organismal fitness, providing a potential mechanism by which mammals overcome unanticipated environmental adversities.

Egr2 is required for the activation of the TH17 pathogenetic program

Yuanyuan Gao, Yan Wang[1], Nicolas Bouladoux[2], Yasmine Belkaid[2], and Vanja Lazarevic[1]

[1]Experimental Immunology Branch, Center of Cancer Research, NCI/NIH[2]Mucosal Immunology Section, Laboratory of Parasitic Diseases, NIAID/NIH

TH17 lineage cells are a heterogenous population of CD4+ effector cells. Some subsets of TH17 cells play an important role in the host defense, while others participate in the pathogenesis of many inflammatory and autoimmune diseases. TH17 cells can alter their differentiation program ultimately giving rise to either protective non-pathogenic or pro-inflammatory pathogenic cells. Molecular switches that control the development of "pathogenic" versus "non-pathogenic" TH17 cells remain largely unknown. In this study, we identified the transcription factor Egr2 as a critical regulator of the TH17 pathogenic program. We found Egr2 was transiently expressed in the early-intermediate stage (24-48 hours) of TH17 differentiation. When ectopically expressed under TH17-polarizing conditions, Egr2 significantly enhanced the expression of TH17 signature genes in a RORγt-dependent manner. Although Egr2 was dispensable for TH17 lineage commitment, its expression was required for the generation of pathogenetic TH17 cells. Mice with T cell-specific deletion of Egr2 were less susceptible to experimental autoimmune encephalomyelitis (EAE) than WT mice. Myelin-specific Egr2-deficeint TH17 cells failed to induce a chronic inflammatory response in the CNS. Transcriptional analysis revealed that Egr2 promoted the pathogenicity of TH17 cells by regulating the expression of pathogenicity-associated genes. Interestingly, Egr2 was not required for the effector function of protective gut-resident TH17 cells in response to Citrobacter rodentium challenge. These findings indicate that Egr2 represents an attractive candidate for the therapeutic targeting of pathogenic TH17 cells while preserving tissue-protective functions of TH17 cells at barrier sites.

Presenter: Gardner, Alycia

Investigating the role of intratumoral dendritic cells in anti-tumor immunity

Alycia Gardner, Álvaro de Mingo Pulido, Brian Ruffell

H. Lee Moffitt Cancer Center

CD8α+ and CD103+ type 1 classical dendritic cells (cDC1s) have been shown to be the dendritic cell (DC) subset critical for inducing a cytotoxic T cell response against tumors. However, while the role for DCs in educating T cells within the lymph nodes is well established, relatively little is known about the impact that DCs have within the tumor. In this study, we made use of orthotopically-implanted tumors derived from C57BL/6 MMTV-PyMT transgenic mice. Bone marrow dendritic cells (BMDC) were generated by ex vivo culture in media containing Fms-like tyrosine kinase 3 ligand (Flt3L). Using these model systems, we show that orthotopically-implanted PyMT tumors are responsive to single-agent checkpoint blockade therapy, indicating the presence of a baseline immune response. Similar decreases in the rate of tumor growth were achieved by systemic administration of Flt3L, and by intratumoral administration of polyinosinic-polycytidylic acid (poly(I:C)), which expand and activate DCs, respectively. Intratumoral administration of BMDCs also significantly slowed tumor growth in C57BL/6 mice, an effect which was further enhanced by co-injection with poly(I:C). In order to examine whether DC trafficking to the lymph node is required for the BMDC-mediated reduction in tumor growth, we used CCR7-/- BMDCs, which do not migrate to the lymph node. Mice treated with these BMDCs exhibited therapeutic response, albeit not to the extent observed with WT BMDC treated mice. These findings suggest a role for DCs locally within the tumor microenvironment, and provide the basis for further investigation of local DC activity in the tumor.

Presenter: Gaudette, Brian

mTORC1 signaling activates a proactive UPR in plasma cell differentiation prior to XBP1 splicing.

Brian Gaudette, David Allman
University of Pennsylvania

The generation of antibody-secreting plasma cells (PCs) from mature, naĬve B cells requires a shift in transcriptional programs leading to striking changes in cellular morphology and physiology. It is thought that the lynchpin of this process is initiation of the unfolded protein response (UPR) through activation of the XBP1 transcription factor downstream of Blimp1-dependent increases in immunoglobulin protein translation. The paradox of a purely reactive, endoplasmic reticulum (ER)-stress-dependent UPR in PC differentiation is the observed lack of PERK-dependent translation inhibition, which is intrinsic to the canonical UPR. By contrasting the UPR activation in two subsets of naĬve B cells with different kinetics of PC differentiation, follicular and marginal zone (MZ) B cells, we are able to differentiate between mTORC1-dependent and ER stress-dependent activation of canonical UPR targets. Our results indicate that MZ B cells, which more rapidly differentiate to PCs, have increased mTORC1 activity at baseline, which is accompanied by increased expression of ER protein folding machinery including negative regulators of PERK activation. This priming of the UPR in MZ B cells is independent of extrinsic activation as it occurs in germ free mice and in mice deficient in TLR and BCR signaling. Furthermore, we demonstrate an mTORC1-dependent increase in expression of canonical UPR targets prior to both Blimp1 and XBP1 activity in naĬve B cells under PC-inductive conditions. Thus, activation of the mTORC1 pathway in early PC differentiation primes the ER to allow for rapid increase in protein production in the absence of canonical ER stress.

Presenter: Gauthier, Kelsey

Intratumoral activation of STING with a synthetic cyclic dinucleotide elicits anti-tumor CD8+ T-cell immunity that effectively combines with checkpoint inhibitors

Kelsey Gauthier, Kelsey Sivick Gauthier, Anthony L. Desbien, Leticia Corrales, Gabrielle Reiner, Laura Hix Glickman, Thomas E. Hudson, Uyen Vu, Natalie H. Surh, Brian Francica, George E. Katibah, Weiwen Deng, David B. Kanne, Justin J. Leong, Ken Metchette, Chudi Ndubaku, Jeffery M. McKenna, Steven L. Bender, Meredith L. Leong, Thomas W. Dubensky, Jr., Andrea van Elsas, and Sarah M. McWhirter

Aduor Biotech Inc

Activation of the STING pathway by intratumoral (IT) injection of synthetic cyclic dinucleotides (CDNs) induces stable tumor regression in pre-clinical models, yet the underlying immune correlates are not fully understood. ADU-S100, a CDN under clinical evaluation, was administered IT with an optimized dosing regimen to explore the immune requirements for anti-tumor efficacy in mouse syngeneic tumor models. We show that CD8+ T cells are necessary and sufficient for durable anti-tumor immunity elicited by ADU-S100 and that activation of STING in hematopoietic cells mediates CD8+ T cell induction. Both type I IFN and TNFa, which are induced by STING pathway activation, influence the anti-tumor immune response. The combination of ADU-S100 and anti-PD1 treatment enhances CD8+ T-cell-dependent non-injected tumor control that correlates with changes in systemic and non-injected tumor-specific T cell responses. Combination of ADU-S100 with checkpoint inhibition also enhances durable immunity in a non-immunogenic tumor model. Together, these results elucidate the immune correlates to STING-mediated anti-tumor efficacy and highlight the potential of combining STING agonists with checkpoint inhibition in the clinic.

Presenter: GLATIGNY, Simon

Pathogenic Th1 and Th17 cells during EAE are influencing regulatory T cells functions

Simon GLATIGNY, Simon Glatigny, Estelle Bettelli

Benaroya Research Institute

Multiple sclerosis (MS) is an autoimmune, inflammatory neurodegenerative disease of the central nervous system (CNS) characterized by demyelination and progressive loss of motor functions. Clinical relapses are thought to be due to multifocal infiltration of immune cells, including Thelper (Th) cells, and B cells, resulting in severe demyelination and ultimately leading to degeneration of neuronal structures. Auto-reactive CD4+ Th cells play an important role in the initiation and progression of MS and its mouse model experimental autoimmune encephalomyelitis (EAE). Pathogenic CD4+ T cells are characterized by specific effector functions and secretion of specific cytokines. Inflammatory T cell functions can be limited effectively by regulatory T cells (Treg). However, Treg from MS subjects show poor suppressive capacities. New research indicates that the function of Tregs on their target is in part due to characteristics of the Treg and the effector T cell (Th1, Th17 or Th GM-CSF) populations to suppress. In recent years, it has been suggested that Tregs function can be compromised under inflammatory conditions and that this effect is due in part to their sensing of proinflammatory cytokines. Among them, IL-6 has been shown to promote the differentiation of Th17 cells and inhibit regulatory T cells. IL-6 signals through a receptor composed of a specific chain (IL-6Ra) and a common Glycoprotein 130 (gp130) chain. Gp130 is a signal-transducing receptor that forms part of the receptor complex for several pro and anti-inflammatory cytokines including IL-6, IL-27, IL-11, LIF and OSM. IL-6R and gp130 have been targeted for the development of improved disease modifying therapies in MS and other autoimmune diseases. However, it is not known whether neutralization of their activity has similar effect on regulatory T cell functions and the development of EAE. The aim of our study was to investigate the role of Treg specific elimination of IL-6R or gp130 and their consequences for the development of EAE. We induced EAE by the adoptive transfer of myelin oligodendrocyte glycoprotein (MOG35-55) specific Th cells into recipient mice with genetic ablation of gp130 or IL6R specifically in Treg (Foxp3Cre Gp130floxed or Foxp3Cre IL6Rfloxed). We show here that the transfer of Th1 cells induce comparable disease in control Foxp3Cre mice and Foxp3Cre IL6Rfloxed mice. In contrast, Foxp3Cre Gp130floxed mice developed exacerbated disease compared to Foxp3Cre IL6Rfloxed mice and control animals. Altogether, our data show that modulating IL-6R and gp130 signaling in Treg has differential effect on their function and on EAE development. Our data suggests that the modulation of IL-6 signaling in Treg does not impact their capacity to control Th1 responses. However, signaling through gp130 promote Treg functions and limit the development of EAE. Future experiments will focus on determining the mechanisms by which gp130 mediated signaling might enhance Treg functions and the progression of EAE and identify which gp130 signaling cytokine(s) can promote Treg activity in the context of EAE and MS.

Presenter: Goretsky, Yitzhar

Epithelial Expression of MHC Class I and T-Cell Fate in the Thymus and Periphery

Yitzhar Goretsky, Author Names separated by commas

University of California, Santa Cruz

Negative selection of developing thymocytes is the major mechanism for developing immune tolerance to self. Self-tolerance also is imposed in peripheral lymphoid tissues by mechanisms that limit the reactivity of mature auto-reactive T cells. We have studied the development and function of T cells expressing the BM3.3 TCR of transgenic CBA (H-2k haplotype) mice. This TCR has high affinity for the alloantigen, H-2Kb. H-2Kb can induce CD8-independent signaling in naĬve BM3.3 T cells. The BM3.3 TCR has high affinity for the alloantigen, H-2Kb. H-2Kb can induce signaling in naĬve 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 T cells also occurs in a double transgenic model in which H-2Kb expression is driven by the guinea pig alpha-lactalbumin promoter (KALxBM3 mice). We have extended those studies to examine in greater detail the development of thymocytes in KALxBM3 mice and to study the functional properties of the T cells that develop in these mice. We also include in these studies another double transgenic line in which H-2Kb expression is driven by the human keratin 14 promoter and the H-2Kb has a GPI tether rather than a cytoplasmic tail (KQxBM3 mice). Flow cytometric analysis of KALxBM3 thymic stromal cell populations showed that the transgenic H-2Kb is expressed by medullary epithelial cells. Levels of expression of H-2Kb in KQxBM3 thymii was too low to identify the thymic stromal cells that express it, but it was detectable by RT-PCR of KQxBM3 RNA. We found that thymocytes from KALxBM3, but not from KQxBM3 mice have diminished numbers of both CD8 SP and CD4 SP thymocytes and the CD8 SP thymocytes express lower levels of CD8 as compared to BM3 and KAL parents. Both KALxBM3 and KQxBM3 have two to four times more DN thymocytes than the parental strains of mice. Using a clonotypic antibody, Ti98, we interrogated the individual populations to determine the fates of thymocytes that either do or do not express the BM3.3 TCR. Apoptosis is low in CD4hiCD8hi DP, DN, and SP thymocytes of KALxBM3 and KQxMB3 mice. Apoptosis of CD4loCD8lo thymocytes is greater in both KALxBM3 and KQxBM3 mice as compared to the parental strains. Late apoptotic thymocytes express high levels of CD5, indicating that they have undergone positive selection. Surprisingly, a higher proportion of Ti98- thymocytes are apoptotic as compared to Ti98+ thymocytes. Interestingly, lymph nodes from the KALxBM3 mice have DN CD3+Ti98+ T cells. These are CD44hiCD62Lhi, the memory phenotype of CD8 T cells. The small percentage of CD8 T cells in KALxBM3 mice is CD8lo. 58.48% of these CD8lo T cells are of memory phenotype (CD44hiCD62Lhi) and of these 30.5% are Ti98hi. This memory phenotype of BM3 TCR-expressing T cells is not observed in BM3 mice, suggesting that it is due to the exposure of KALxBM3 to H-2Kb. KALxBM3 mice are less effective at rejecting KAL skin grafts than are BM3 mice. Rag-deficient BM3 mice reject KAL skin, indicating that H-2Kb-specific BM3 T cells (and not T cells with other TCRs that recognize H-2Kb peptides presented on self MHC) mediate the rejection. An interesting question is how the H-2Kb molecule whose expression is restricted to epithelial cells get presented to T cells in draining lymph nodes of skin graft recipients. One possibility is that epithelial cells produce H-

2Kb-bearing exosomes, which are then acquired by dendritic cells that then present the H-2Kb to T cells. Analysis of exosomes derived from KALxBM3 epithelial cells do not support this model. In summary these studies show that expression of H-2Kb whose expression in the thymus is restricted to epithelial cells affects the efficiency of negative selection of H-2Kb-specific T cells and that these T cells are not fully tolerized to H-2Kb-bearing skin grafts. Future studies are focused on understanding the mechanisms of epithelially expressed H-2Kb presentation to mature peripheral T cells.

Presenter: Gupta, Sneh Lata

A subset of CD73 expressing IgM+ antigen-experienced cells is dependent on T-B interaction.

Sneh Lata Gupta, Lucas D'Souza, Vineeta Bal, Satyajit Rath, Anna George

National Institute of Immunology

Memory B cells in the murine system are composed of Switched memory and IgM memory. The maturation markers CD73, CD80 and CD273 have previously been used to characterize multiple phenotypically and functionally distinct populations within this compartment. The factors involved in the formation of these subsets, however, remain unclear. We report that a subset of CD73 expressing IgM+ antigen-experienced cells with memory characteristics is diminished in T-cell deficient mice and provision of T cells by adoptive transfer or in mixed bone marrow chimeras rescued this deficiency. We also identify a role for CD40-CD154 signaling in this phenomenon, as CD73+ subsets were reduced in CD40-/- mice and also in the CD40-/- compartment of mixed-marrow chimeras. In the absence of CD40-CD154 signaling, we find CD73 expression to be reduced in splenic T cells and Age-associated B cells (ABCs), suggesting the importance of T-B interactions in the induction of this marker.

A systematic approach to characterizing CD4 T cell function in Borrelia burgdorferi infection

Elizabeth Hammond, Kimberly Olsen, Nicole Baumgarth

University of California Davis

Borrelia burgdorferi is a spirochete bacteria that is the causative agent of Lyme disease. It is widely accepted that B. burgdorferi has evolved a wide variety of mechanisms to subvert host immune responses, which leads to persistent infection. Our lab has previously demonstrated that T-dependent B cell responses are impaired in a murine model of B. burgdorferi infection. Specifically, we observed structural defects in germinal centers (GCs), early collapse of GCs, and impaired formation of long lived plasma cells and memory B cells. In light of other studies that demonstrated the ineffectiveness of T cells in providing protection against B. burgdorferi, we have developed the hypothesis that the CD4 T cell response is impaired during B. burgdorferi infection, thus contributing to spirochete persistence. In order to systematically determine which aspects of the CD4 T cell response are impaired during B. burgdorferi infection, we first identified an immunodominant T-dependent antigen, arthritis related protein (Arp). Furthermore, we determined a highly immunogenic Arp epitope in C57BL/6 mice. Using the identified Arp epitope, we developed an I-Ab restricted tetramer in order to track an antigen-specific CD4 T cell response using flow cytometry. Furthermore, we developed a FACS staining protocol to identify follicular T helper cells (TFH) which are localized in the GC. Using these reagents and techniques, we tracked the CD4 T cell response over the course of acute infection with B. burgdorferi.

Determining the effect of diverse host-associated microorganisms on tolerance induction

Erika Hayes, Minh Pham, Marc Gavin, Daniel Campbell

Benaroya Research Institute

Regulatory T cells (TR) are required for maintaining immune homeostasis and preventing loss of tolerance to self-antigens. Therapeutic manipulation of TR for induction or restoration of tolerance has been pursued for treatment of autoimmunity, but while it has been relatively easy to induce tolerance in specific pathogen free (SPF) mice, this has not translated to human patients. It was recently reported that diverse host-associated microorganisms are acquired by traditional SPF mice through co-housing with pet store mice. This cohabitation dramatically altered the SPF mouse immune phenotype and response to antigenic challenge, ultimately inducing an immune transcriptomic signature akin to that of an adult human. We have found that co-housed mice have an enriched inflammatory dendritic cell (DC) population in the spleen, as well as a ratio of effector to regulatory T cells that is skewed heavily towards effector lymphocytes. We hypothesize that the increased lymphocyte activation and effector/memory phenotypes in CH mice are a product of increased DC activation. Based on evidence that antigen-experienced T cells can reciprocally affect DC responses, we further predict that the Teff-skewed CD4+ T cell compartment will perpetuate DC activation, leading to a less tolerant immune state. As this co-housing model is able to better recapitulate the heightened basal activation status of lymphocytes observed in humans, utilization of this model to evaluate key factors for tolerance induction will be informative on why efforts to translate therapies from SPF mice to humans have failed. For example, when antigen-specific immunotherapy (ASI) is administered in SPF mice, it results in efficient induction of TR, but this is abrogated in the presence of an inflammatory stimulus. These studies will give evidence for use of more realistic pre-clinical animal models to provide better predictive power for results of immune targeted therapies in humans, and reveal important parameters for induction or restoration of tolerance that will aid in designing therapeutics.

Antigen recognition by CD8+ T cells in non-lymphoid tissues initiates a transcriptional program of tissue-resident memory differentiation

Samuel J. Hobbs, Jeffrey C. Nolz

Oregon Health and Science University

Tissue-resident memory (TRM) CD8+ T cells permanently reside in non-lymphoid tissues where they are important mediators of both host defense and inflammatory disease. TRM cells are derived from effector cells after they have been recruited into non-lymphoid tissues, but the molecular and transcriptional mechanisms that control TRM differentiation are largely undefined. We have recently used a model of localized epicutaneous infection with Vaccinia viruses (VacV) expressing individual model antigens to demonstrate that local antigen recognition in the skin microenvironment enhances TRM formation by ~50-100 fold. To understand how T cell receptor (TCR) engagement may promote TRM differentiation, we sought to identify effector CD8+ T cells that were actively recognizing cognate peptide-MHC in vivo. Using an endogenous interferon-gamma (IFNγ)-YFP reporter, we found that only ~20% of the antigen-specific CD8+ T cells in the skin microenvironment produce IFNγ during the course of a VacV skin infection. However, genome-wide transcriptional profiling revealed that the T cells producing IFNγ in the skin are also undergoing dramatic changes in gene expression that resemble mature TRM cells. In fact, effector CD8+ T cells producing IFNγ also expressed the transcription factor Blimp1, which has previously been shown to be critical for the acquisition of the core TRM transcriptional profile. We further show that TCR stimulation is sufficient to induce Blimp1 expression in effector, but not naĬve CD8+ T cells, suggesting that antigen recognition by effector T cells results in unique transcriptional consequences not observed during the initial activation of naïve T cells. Together, these studies demonstrate that antigen recognition by effector CD8+ T cells in non-lymphoid tissues is a critical factor impacting gene expression, which ultimately promotes the differentiation of TRM cells during viral skin infection.

Investigating lipid-metabolic requirements in anti-inflammatory macrophage activation

Wei-Yuan Hsieh, Tin Doung, Kevin Williams, Quan Zhou, Viet Bui, Joseph Argus, and Steve Bensinger

Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles

Macrophages are immune cells responsible for a broad range of processes such as killing invading pathogens, clearing apoptotic cells and subcellular debris, initiating wound-healing programs, and presenting antigens to T cells. To achieve these different functions, macrophages possess the capacity to acquire pro- and anti-inflammatory programs by altering fluxes through various metabolic pathways. It is well understood that interferon (β/γ) and specific TLR agonists suppress macrophage lipid synthesis to promote classical-proinflammatory activation. However, little is understood how anti-inflammatory polarizing signals, such as IL-4, coordinate macrophage lipid-metabolic reprogramming. Here, we show that IL-4 signaling co-opts the activation of SREBP-SCAP pathway in bone marrow derived macrophages (BMDMs) to induce lipid synthesis. M(IL-4) polarization is significantly impaired in the absence of SCAP. 13C tracer analysis of fatty acid methyl esters (FAMEs) of M(IL-4) revealed the accumulation of specific long chain fatty acids that are required for macrophage polarization. The location of these fatty acids in complex lipids is further characterized through the use of lipidomics. These results provide a new role for lipid metabolism in supporting macrophage activation and function.

Light-based screening for determining genetic and transcriptional underpinnings of cellular heterogeneity

Kenneth Hu, Matthew Krummel
UCSF Department of Pathology

ABSTRACT TEXT NOT AVAILABLE ONLINE

Presenter: Huang, Xin

The role of CCR5 in the maintenance of intestinal immune homeostasis

Xin Huang, Daniel T. Utzschneider, Arnaud Delpoux, Chenyen Lai, Steve M. Hedrick

1Molecular Biology Section, Division of Biological Sciences, UC San Diego, La Jolla, CA 92093 2Cellular and Molecular Medicine, UC San Diego, La Jolla, CA 92093

The intestinal epithelium provides a crucial barrier against external pathogens. Disruption of this intestinal barrier leads to microbial translocation, compromised immune surveillance and thus can result in systemic infections. Specialized immune cells play an important role in maintaining intestinal integrity and microbial homeostasis. In order to analyze the impact of individual cell populations on sustaining the intestinal integrity, we created a novel mouse model, which allows us to identify, sort, or specifically delete individual cell subsets based on the expression of the chemokine receptor type 5 (CCR5). In this mouse model, we replaced the coding region of CCR5 with a cDNA coding primate diphtheria toxin receptor (pDTR) and eGFP fusion, so that the altered allele constitutes a CCR5 null and renders the cells carrying this allele sensitive to non-inflammatory apoptosis via injected diphtheria toxin(DT). Here, we showed that administration of DT lead to a complete loss of NK cells as well as a partial loss of NKT cells, CD44+CD4 cells, CD44+CD8 cells, gdT cells and CD11c+ dendritic cells. Interestingly, sustained DT-treatment induced dramatic weight loss, hypothermia and ultimately mortality, which we found was due to the loss of hematopoietic cells. Finally, the administration of a broad-spectrum antibiotic cocktail prevented the occurrence of illness, indicating its dependency of gut integrity. All together, we generated a novel and flexible mouse model in which CCR5+ cells can be identified, sorted, or killed with the administration of DT. Our findings characterized the key importance of CCR5 on different immune cell subsets and suggest that CCR5+ immune cells play an essential role in intestinal immune homeostasis on several levels.

Cell death without the mess: Understanding specific contributions of cell death programs in shaping immunity.

Nicholas Hubbard, Andrew Oberst, Annelise Snyder, Michelle Messmer

University of Washington

Inflammatory and non-inflammatory forms of programmed cell death (PCD) such as necroptosis, pyroptosis and apoptosis has been increasingly indicated a key player in the development of pathogenic and protective immune responses, particularly in privileged environments such as the gut, lung and skin. Notably, each of these cell death programs is hypothesized to yield distinct immune consequences due unique features intrinsic to each process resulting in unique cytokine, chemokine and DAMP profiles. Despite these implications, programmed cell death in disease settings occurs downstream of complex, pleiotropic signals such as viral, bacterial or fungal infection, complicating our understanding of the specific effects of cell death on the immune response. To address this question directly, we have developed a novel system, for rapid and specific induction of 'pure death' via necroptosis, pyroptosis or apoptosis using constitutively active forms of the cell death effectors Caspase-9 (apoptosis), RIP3K (necroptosis) or Caspase-1 (pyroptosis). When delivered to target tissue using recombinant adeno-associated virus (AAV), these proteins induce rapid, specific, controlled in vivo cell death. Further, we extend this approach to selectively activate the individual molecular events contained within each cell death program, allowing us to further dissect specifically how each process contributes to the downstream immune response, focusing initially on the lung as a target organ. Programmed cell death in the lungs represent a challenge due to their extensive contact with the outside environment, variety of immune challenges and intolerance to damage. Highlighting this, damage the lung epithelium by a variety of allergens, environmental pollutants and respiratory infections has been linked to the development of asthma and allergy, leading us to predict that multiple forms of programmed cell death mechanisms are relevant in this in this tissue. We hypothesize that activation of different cellular death pathways in the lung epithelium will differentially impact innate and adaptive immunity, and that this contributes to both appropriate and inappropriate host responses.

Presenter: Johar, Shamael

Evaluation of Hibiscone C derivatives in the inhibition of Phosphatidylinositol-3-Kinase in T cellsEnter a Title for your Abstract

Shamael Johar, Caroline Besley, Brian C. Goess, Jason S. Rawlings

Furman University

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, while a nonfunctional PI3K can result in immunodeficiency. 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 , suggesting that hibiscone C shares similar biological activities to wortmannin. 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 carbonyls of the furan ring would affect potency of the molecule. Hibiscone B, hibiscone B diastereomer, AU and AUD all show reduced inhibitory activity compared to Hibiscone C, suggesting that while these carbonyls are important for maximal binding to PI3K, they are not absolutely required to achieve inhibition. These findings provide avenues for further manipulations of Hibiscone C functional groups to maximize efficacy of the molecule.

Presenter: Joseph, Chelsea

Mechanisms of Activation- Induced Chromatin Decondensation in the Lymphoid Lineage

Chelsea Joseph, Morgan Fretwell, Sierra McDonald, Alynna Knaub, and Jason S. Rawlings
Furman University

It has previously been demonstrated that chromatin decondensation is required for aß T cell proliferation. B cells, ?d T cells, and NK cells share the same lineage as aß T cells, originating from a common lymphoid progenitor. However, these different cell types are known to proliferate at different rates upon activation. In this study, we investigated the hypothesis that chromatin decondensation regulates proliferation of cells of the lymphoid lineage. We found that in their basal (naïve) state, each cell type of the lymphoid lineage experiences a different state of chromatin decondensation, which correlates with their known ability to proliferate in response to activation stimuli: both NK cells and ?d T cells are more decondensed than aß T cells while B cells have similarly condensed chromatin. We also investigated the effects of activation on the rate of decondensation in B cells and ?d T cells. We show that Lipoplysaccharide, a known activator of B cells, induces the decondensation of chromatin in B cells. To determine if both T and B cells can decondense chromatin at the same rate, both were stimulated with PMA and Ionomycin. B cells and aß T cells decondensed chromatin in a similar manner, although there appears to be differences in kinetics between the two. Interestingly, ?d T cells decondense less than aß T cells when activated suggesting that ?d T cells may potentially utilize a different mechanism. Recently, our lab demonstrated that calcium regulates chromatin decondensation in aß T cells; here, we show that calcium also regulates decondensation in B cells, suggesting that B cells utilize a similar mechanism for decondensation. Future studies will investigate if calcium can also regulate the decondensation of ?d T cells and NK cells, establishing a conserved mechanism to control proliferation of the lymphoid lineage.

Presenter: Kastenschmidt, Jenna
A role for group 2 innate lymphoid cells in muscular dystrophy
Jenna Kastenschmidt, Ileen Avetyan, Rayan Yahia, Rachel Ayer, S. Armando Villalta
University of California, Irvine

A recently identified innate lymphocyte population - group 2 innate lymphoid cells (ILC2s) - were shown to be critical regulators of type II immune responses and tissue repair. Given that type II immune responses are required for efficient skeletal muscle regeneration, we sought to test the hypothesis that ILC2s promote muscle regeneration by regulating type II inflammatory responses in mdx mice. The mdx mouse is a model of Duchenne muscular dystrophy (DMD) that is characterized by cyclic bouts of muscle degeneration and regeneration. We show that ILC2s are activated, reflected by increased numbers in dystrophic muscle and elevated expression of IL-5. IL-2/anti-IL-2 complex (IL-2c) and IL-33 effectively increased the number of ILC2s and expression of type 2 cytokines (IL-5 and IL-13), indicating that ILC2s are capable of promoting type II immune responses in dystrophic muscle. Additionally, we found IL-2c/IL-33 treatment increased myofiber cross sectional area, suggesting enhanced muscle regeneration. Collectively our data support a working model in which ILC2s promote type II immune responses in dystrophic skeletal muscle to promote regeneration.

Uptake of tumor-antigen induces myeloid cell reprogramming in lung metastasis

Kelly Kersten, Ran You, Mark Headley, Sophia Liang, Matthew F. Krummel
Department of Pathology, University of California San Francisco

Metastatic spread is one of the deadliest aspect of cancer. Yet little is known about the fate of disseminated tumor cells when they arrive in and colonize distant organs, or how they interact with the immune system during the early steps of metastasis. Previously, our laboratory has developed a model for stable intravital two-photon lung imaging in mice to capture the early interactions of disseminated tumor cells with host immune cells during pulmonary metastasis1. We showed that soon after they enter the lung tissue, tumor cells continuously shed microparticles or cytoplasts in the lung capillaries that move independently along the lung vasculature1. Distinct populations of myeloid cells ingested these tumor microparticles and sequentially accumulate in the lung interstitium, possibly providing a supportive niche for the outgrowth of metastases. Although the numbers of myeloid cell subsets rise globally in the early metastatic lung, gene expression analysis of macrophages - the most predominant tumor particle-ingesting myeloid population - revealed that ingestion of tumor particles results in a phenotypic switch. However, the functional significance of this phenotype change remains unclear. Here we show that macrophages that have ingested tumor particles down-regulate markers including Flt3, Btla and Zbtb46 that are associated with "dendritic cell maturation", suggesting a reduced capacity to activate anti-tumor T cell responses. In addition, genes associated with "cell adhesion and extracellular matrix pathway" were upregulated in tumor-ingested macrophages compared to non-tumor-ingested macrophages. In vitro co-cultures of bone marrow-derived macrophages and B16.ZsGreen tumor cells recapitulated our in vivo findings and revealed that the upregulation of VCAM, CD63 and CD38 in macrophages occurs rapidly after ingestion of ZsGreen+ tumor particles. Moreover, Ingenuity pathway analysis revealed enrichment of mTOR signaling - a major regulator of cell metabolism - in tumor-ingesting macrophages. In vivo blockade of mTOR signaling with rapamycin abrogated the upregulation of VCAM, CD63 and CD38 in ZsGreen+ macrophages, suggesting that the phenotypic change observed in tumor-ingesting macrophages might be mTOR-dependent. Future experiments are focused on deciphering the functional significance of the phenotypic switch of macrophages after ingestion of tumor material. These results will increase our understanding of the interactions between disseminated tumor cells and immune cells, and will eventually help the development of novel immunotherapeutics to prevent metastatic disease. 1. Headley, M. B. et al. Visualization of immediate immune responses to pioneer metastatic cells in the lung. Nature 531, 513-517 (2016).

Presenter: Kilgore, Augustus

IL-27p28 production by DCs predicts adjuvant-elicited CD8+ T cell responses

Augustus Kilgore, Seth Welsh, Benjamin J. Kedl, Nathan D. Pennock, Christopher A. Hunter, Ross M. Kedl

University of Colorado Denver Anschutz Medical Campus, School of Medicine, Department of Immunology and Microbiology

Generating effective T cell responses to subunit immunization is one of the major unsolved challenges of vaccine development. Attempts to address this challenge have succeeded in creating robust inflammatory environments but have failed to produce the level of T cell response needed for protective or therapeutic immunity. We previously reported that T cell responses induced by subunit vaccination were dependent on the cytokine IL-27. Using a novel IL-27p28-GFP reporter mouse, we show here that the degree to which an adjuvant induces IL-27p28 production from dendritic cells and monocytes directly predicts the subsequent magnitude of the T cell response. These data are the first to identify a concrete innate correlate of vaccine-elicited cellular immunity. Of note, our reporter indicates that IL-27 production by DCs in response to adjuvant administration is limited to 6-18 hours post vaccination, a time frame that is highly amenable to evaluation by multiphoton microscopy. We have thus utilized this technique to evaluate by live imaging the influence of IL-27 on T cell localization, migration and interaction with DCs during the earliest periods of the subunit vaccine-induced T cell response. Our data continue to support the assertion that divergent biological mechanisms guide subunit vaccine-elicited immunity versus infection-generated immunity.

Presenter: Kumar, Sushil

PD-L1 expressing myeloid cells limit response to CSF-1R targeted therapy

Sushil Kumar, Collin M. Blakely, Dharini Murugan, Patrick Flynn, Takahiro Tsujikawa, Reid Goodman, Dario Barbone, Raphael Bueno, David J. Sugarbaker, David M. Jablons, V. Courtney Broaddus, Brian L. West, and Lisa M. Coussens

Oregon Health & Science University

Malignant mesothelioma (MM) is an inflammation-associated cancer that is markedly resistant to "standard-of-care" chemotherapy with extremely poor prognosis. We report herein that chemotherapy leads to increased frequency of colony stimulating factor-1 receptor (CSF-1R) + macrophages in human MM, as well as in immunocompetent mouse model of MM. While therapeutic blockade of CSF-1R instigated activation of CD8+ T cells in primary tumors resulting in ~50% decreased primary tumor burden, combined therapy did not improve animal survival as therapy failed to reduce frequency or burden of pulmonary metastases. To investigate mechanisms underlying the tissue-specific response to combined chemo/CSF1R-inhibition, we screened inhibitory immune checkpoints by flow cytometry in primary and metastatic tumor tissue that led to the identification of programmed death ligand 1 (PD-L1) as an abundant immune checkpoint molecule expressed by tumor associated macrophages in primary tumors and alveolar macrophages in metastatic lungs. Based on this, we hypothesized that combination blockade of PD-L1 with CSF-1R antagonist would improve survival by enhanced CD8+ T cell activation and cytotoxicity in both primary and metastatic tumors, thereby extending survival and improving outcome. Indeed, combination therapy using CSF-1R/PD-L1 inhibitors increased activated CD8+ T cell frequency and further reduced tumor burden at both primary and metastatic sites resulting in increased animal survival. Interestingly, ICOShi CD4+ T cells identified tumor microenvironments responsive to checkpoint blockade for CD8+ T cell expansion. To determine if improved CD8+ T cell-dependent outcome reflected antigen-dependent clonal expansion in situ or peripherally, we evaluated clonal characteristics by T cell receptor (TCR)β sequencing of PBMCs, primary and metastatic tumors. This analysis revealed significant clonal expansion of T cells by in combination-therapy groups (CTX, CSF-1R antagonist, αPD-L1 mAb) and hinting towards enrichment of PD-1hi T cells in primary and metastatic tumors, which is corroborated by flow cytometry-based evaluation of CD8+ T cell repertoires in both tumor niches. CD4+ T cell helper function was necessary for CD8+ T cell expansion by checkpoint blockade, that was facilitated by CSF1-R inhibition in primary tumors. CD8+ T cell expansion was essential for efficacy of CSF-1R/PD-L1 inhibitors as cancer therapy since depletion of either CD8+ T cells or CD4+ T cells subverted therapy response. These data demonstrate that tissue-specific tumor niches pose distinct requirements for generating adaptive immune responses and/or perturbing/abrogating tumor growth. Results from these studies highlight a rationale for combination therapy where individual constituents target different immunosuppressive microenvironments to impact BOTH primary tumor responses as well as thwarting secondary tumor growth.

Presenter: Le, Catherine

Diet-induced obesity impairs induction of Experimental Autoimmune Encephalomyelitis and is restored by PD-1 blockade

Catherine Le, Lam Khuat, Athena Soulika, and William J. Murphy

University of California, Davis

There is an increasing prevalence of obesity in the U.S. reaching pandemic proportions. Obesity has marked effects on immune function. Increased adiposity and accumulation of visceral fat results in continuous, low-level systemic inflammation that induces or exacerbates several chronic conditions. Obesity is associated with increased susceptibility to infection and decreased adaptive immune response to vaccination. We were interested in examining the effects of obesity on adaptive autoimmune immune responses using a mouse model of diet-induced obesity. The pathogenesis of experimental autoimmune encephalomyelitis (EAE), a model for autoimmune demyelinating disease of the central nervous system (CNS) such as multiple sclerosis, is mediated by autoreactive CD4+ T cells, particularly Th1 and Th17 subsets. We immunized 8-month-old C57Bl/6 male mice who were fed an open-source purified diet consisting of either 60% fat or 10% fat as a control diet starting at 8 weeks of age at which time there was notable differences in accumulated body fat. The groups were then immunized with 300 μg of rodent MOG35-55 peptide in complete Freund's adjuvant (CFA) containing 5 mg/ml heat-killed Mycobacterium tuberculosis, which has been demonstrated to induce EAE symptoms and pathology in susceptible strains. Lean mice had significantly greater body weight loss compared to diet-induced obese mice on day 12 (22% vs 10% body weight loss) and day 13 (28% vs 11% body weight loss) post-induction. Lean mice also exhibited earlier onset of severe clinical symptoms of hind limb paralysis by day 10 post-induction. Clinical scores were correlated with greater number of MOG-specific tetramer-positive CD4 T cells and greater number of IFN-gamma and IL-17a-expressing CD4 T cells in the CNS of lean mice. Diet-induced obese mice eventually developed clinical symptoms comparable to lean mice with a 4-day delay in the induction of severe clinical symptoms. Decreased functional cytokine expression called into question the exhaustive nature of T cells in obese mice through the up-regulation of the PD-1/PD-L1 signaling, a pathway that has been implicated as a negative regulator of EAE. We induced lean and obese mice with EAE and treated with anti-PD-1 blockade at 500 μg on Day 0 and 250 μg on Day 2, 4, 6, and 8. The delay in induction was partially rescued by PD-1 blockade treatment, resulting in earlier and faster induction of EAE in obese mice in both clinical score and number of pathologic CD4 T cells in the CNS. The kinetics of induction did not differ with anti-PD-1 blockade in lean mice. Our findings may indicate an impairment of the adaptive arm of the immune system through the PD-1/PD-L1/PD-L2 pathway in obesity, indicating an augmentation of the checkpoint pathway in settings of systemic inflammation and autoimmunity.

Microanatomical organization of CD4+ T cell responses to vaccines

Joseph Leal , Brandy Olin, Karan Kohli, Michael Gerner
Department of Immunology, University of Washington

The cellular immune response to foreign antigens is orchestrated through interactions between innate antigen presenting cells, dendritic cells (DCs), and T lymphocytes. Activated T cells are polarized into distinct helper cell subsets, promoting generation of highly tailored protection to distinct pathogens. Further, multiple subsets of DCs exist with non-overlapping functions with respect to T cell activation and effector cell differentiation. Utilizing a novel quantitative imaging approach, our laboratory has shown that these DC subsets occupy different spatial locations within secondary lymphoid tissues during the steady state. Here we investigated whether the spatial localization of DC subsets influences T cell priming using mouse models of immunization. As anticipated, distinct vaccine adjuvants elicited divergent programs of T cell effector differentiation. Intriguingly, we found that different adjuvants also induced divergent spatial patterning of DCs and CD4+ T cells in draining lymph nodes, and that such positioning correlated with effector T cell polarization. Ongoing and future studies will examine the hypothesis that distinct adjuvants induce formation of discrete tissue microenvironments that are functionally specialized in generating specific programs of T cell immunity.

Presenter: Lee, Jinwoo

Origin and Potentiation of Tissue-Resident Type 2 Innate Lymphoid Cells

Jinwoo Lee, Christoph Schneider, Jesse C. Nussbaum, Hong-Erh Liang, Roberto Ricardo-Gonzalez, Steven Van Dyken, Lucas K. Smith, Saul A. Villeda, Richard M. Locksley

University of California, San Francisco

ABSTRACT TEXT NOT AVAILABLE ONLINE

Presenter: Lema, Diego

Identification of Mechanisms of Immune Regulation and Exhaustion in Patients with Advanced Non-Small Cell Lung Cancer

Diego Lema, Jankowska-GanÂ E, Burlingham W, Leal T
University of Wisconsin-Madison, Dept of Surgery, Transplant Division

Background: The success of PD-1/PD-L1 inhibition is clearly dependent on optimal patient selection. Developing validated biomarkers that identify patients with an increased probability of response to these antibodies remains a challenge. DTH responses are a standard method to evaluate cellular immune responses in animal and human immunotherapy trials. The trans-vivo delayed-type hypersensitivity (tvDTH) methodology, using the SCID mouse footpad swelling response to human PBMC (peripheral blood mononuclear cells) + antigen injection, and using antibodies to block the regulatory pathways, was developed in our laboratory and has been used to characterize regulation systems in tolerant transplant recipients and more recently in prostate cancer. Methods: In this pilot project we aim to investigate the mechanisms of regulatory immune responses in patients with advanced NSCLC receiving anti-cancer therapies using the tvDTH, and to correlate tvDTH results with responses to checkpoint inhibitor blockade therapy. So far we have enrolled 4 NSCLC patients. PBMCs are obtained by blood draw and lung cancer antigen (LCA) is prepared from malignant pleural effusion cellular pellet or fresh frozen tumor. 1 of 4 patients' sample was negative for malignancy Results: Our preliminary data suggests that all NSCLC patients with pleural effusions positive for malignancy had a PD-1-dependent exhausted immune response towards their LCA. Remarkably, 1 of these patients not only showed PD-1-dependent exhaustion, but also PD-1-dependent bystander suppression, whereby a positive control immune response was reduced by co-injection of LCA, suggesting the existence of immunosuppressive mechanisms. Unlike our previous results with prostate cancer, no patient showed CTLA-4-dependent exhaustion or suppression. Importantly, an antigen preparation from a NSCLC that showed no malignant cells by cytology did not induce a swelling response. Conclusions: We plan to expand our study patient population and follow up our patients in order to correlate tvDTH results with clinical outcomes.

Regulation of tissue-resident macrophage development by IL-7R signaling

Gabriel Leung, Taylor McCann, Camilla Forsberg, Anna Beaudin
University of California, Merced; University of California, Santa Cruz

In addition to their duties in immune surveillance, specific tissue-resident macrophages play critical roles in normal tissue homeostasis and disease. Many populations of tissue-resident macrophages derive from fetal progenitors and self-maintain across the lifespan through in situ proliferation, independent of bone marrow hematopoiesis. However, the developmental mechanisms that specify the unique function of fetal-derived tissue-resident macrophages is poorly understood. Here we have identified a novel cytokine regulating tissue-resident macrophage development using an IL7Ra-cre lineage tracing model. Adult tissue resident macrophages in the brain (microglia), skin (Langerhans cells), liver (Kupffer cells), and lung (alveolar macrophages) were extensively labeled by IL7ra-cre, in the absence of IL7ra message or protein expression. To gain insight into developmental expression of IL7ra, we profiled IL7Ra surface expression, mRNA expression, and IL7ra-cre-driven labeling during fetal myeloid development. During fetal liver-stage hematopoiesis, macrophages derived from the yolk sac are thought to already be present in the tissues but may be replaced or replenished by incoming fetal liver-derived monocytes. We observed rapid upregulation of IL7ra surface expression during a limited window of tissue macrophage establishment, and dynamic regulation of IL7ra message and protein levels in monocyte precursors, suggesting that IL7ra regulated the transition from fetal liver monocytes into tissue macrophages. Blockade of the IL7R using an antagonistic monoclonal antibody significantly decreased cellularity of lung and skin tissue-resident macrophages at birth, with a concomitant increase in the number of fetal liver monocytes. These data suggest that during late gestation, IL7Ra is upregulated as fetal monocytes exit the fetal liver and differentiate into macrophages, and also provides evidence that IL-7R signaling directly regulates fetal tissue-resident macrophage development. Ongoing work addresses the function of IL-7 signaling in myeloid development, in vivo and ex vivo, as well as the distinct function of IL-7R-marked macrophages in adult immunity.

Presenter: Lieberman, Nicole

Macrophage-based immune therapy for DIPG

Nicole Lieberman, Heather Kovar, Amira Davis, Jeff Stevens, Conrad Winter, Courtney Crane
Seattle Children's Research Institute

Diffuse Intrinsic Pontine Glioma (DIPG) is a universally fatal tumor in children, with a median survival of only 10 months. Due to its location in the brainstem, surgical resection is not an option. Immunotherapy is a particularly appealing option to treat DIPG because of its potential to specifically eliminate tumor cells while leaving healthy tissue intact. However, little is known about the tumor microenvironment (TME) in DIPG, and its effect on the immune system is unclear. We have examined tumor microarrays of DIPG and found reduced infiltration of myeloid cells and CD4+ and CD8+ T cells relative to low- and high-grade pediatric glioma. This is consistent with a Nanostring nCounter analysis of immune-related transcripts in non-tumor, DIPG, LGG, and HGG brain, which reveals less expression chemokines that attract myeloid cells, such as MCP-1, MCP-2, and MCP-3, present in DIPG tissue. However, relative to the expression of CD68, a pan-macrophage marker, DIPG tissue contains more of some costimulatory molecules (CD86, CD40) and MHC class II than does HGG tissue, suggesting that the TME of DIPG may be less immunosuppressive than that of HGG. Therefore, we are investigating the use of adoptively transferred macrophages to stimulate an antitumor immune response in DIPG, including priming NK and CAR T cell anti-DIPG cytotoxic activity.

Regulation of TLR Signaling by Integrin [a]v[b]3 in Plasmacytoid Dendritic Cells

Alina Lorant, Adam Lacy-Hulbert

University of Washington

Systemic lupus erythematosus (SLE) is characterized by the production of autoantibodies against nucleic acids and associated nuclear proteins. The nucleic acid-sensing Toll-like receptors (TLR7/9) have been heavily implicated in the development of SLE in humans and in lupus-like autoimmunity in mouse models, suggesting that SLE is triggered by innate immune responses to self-derived nucleic acids. In addition, SLE is strongly associated with high levels of type I interferon (IFN), largely produced by plasmacytoid DCs (pDCs) after TLR7/9 stimulation, contributing to autoimmunity. Understanding how pDC production of IFN is regulated, and how this breaks down during autoimmunity and other inflammatory disorders, is an important unanswered question. Although TLRs function as critical sensors of pathogens through recognition of microbial, fungal and viral ligands, TLR signaling also relies on a wide range of co-receptors and accessory proteins, which present ligands for TLR binding, amplify signaling, and promote internalization of ligands to endosomal compartments where they can engage endosomal TLRs. As an example, engagement of pDC Fc receptors by DNA-containing immune complexes promotes endosomal maturation, allowing activation of IRF7 and production of IFN downstream of TLR9. I hypothesize that co-receptors for self-derived TLR ligands may likewise regulate TLR signaling in pDCs, promoting 'tolerogenic' responses, and that loss of these regulatory signals will predispose to autoimmunity. This hypothesis is supported by two major findings from many laboratories, including my mentor Dr. Lacy-Hulbert. First, engagement of apoptotic cells, a likely source of autoantigens in SLE, inhibits pro-inflammatory cytokine production and promotes development of a regulatory phenotype in macrophages or DCs, characterized by production of IL-10 and TGF-β. Second, recognition and phagocytosis of dying cells is impaired in SLE, and disruption of this process in mice leads to SLE-like autoimmune disease. However, the exact mechanisms underlying this process, and whether they operate in pDCs, remain unclear. Recently, my mentor's laboratory has identified a novel role for αvβ3 integrin in regulating TLR signaling in B cells, through a mechanism involving endosomal maturation and non-canonical autophagy. αvβ3 and the related integrin αvβ5 serve as internalization receptors for apoptotic cells, and we therefore speculate that this represents a mechanism to regulate TLR signaling in response to self-antigens derived from dying cells. Supporting this, deletion of αv or β3 integrins in B cells results in increased production of autoantibodies. In this project, I will investigate how αv integrins function to regulate TLR signaling and IFN-I in pDCs first in vitro, and then in the context of autoimmunity. So far I have seen altered cytokine responses to TLR9 stimulation in αv-KO cells compared to wild-type. Decreased interferon responses in αv-KO mice suggest the integrin could be limiting pDCs response to when self-derived stimuli are present.

Presenter: Marcovecchio, Paola

Kindlin-3 is required for nonclassical monocyte patrolling and enables protection of lung tissue from metastatic seeding of B16 melanoma.

Paola Marcovecchio, Tacke R, Hanna R, Wu R, Yoakum D, McArdle S, Liikanen I, Goldrath AW, Ley K, Hedrick CC

La Jolla Institute, UCSD, MedImmune, Fate Therapeutics

Nonclassical monocytes are effective in preventing metastatic tumor cells from seeding in lung tissue. Nonclassical monocytes patrol the vasculature to aid in maintaining vascular homeostasis and to respond to inflammatory signals. However, whether patrolling is required for nonclassical monocytes to prevent tumor metastasis is unclear. Using an inducible cre-lox mouse model and intravital microscopy, we show that Kindlin-3, an intracellular adaptor protein capable of activating integrins to their high-affinity conformation, is essential for driving the patrolling activity of nonclassical monocytes. Without Kindlin-3, nonclassical monocytes cannot patrol along the vascular endothelium, and are only able to roll and occasionally arrest. We hypothesized that nonclassical monocytes require patrolling to prevent metastases to the lung. When B16 melanoma tumor cells were injected intravenously into wild-type mice or mice with Kindlin-3 deficient monocytes, there was a significant 4-fold increase in B16 tumor metastases in the lungs of Kindlin-3 deficient mice compared to wild-type mice. Kindlin-3-/- nonclassical monocytes showed defective migration to the lung after intravenous tumor cell injection. Further investigation revealed that Kindlin-3-/- nonclassical monocytes failed to take up tumor material compared to wild-type monocytes. We found that this was due to lack of integrin activation in the absence of Kindlin-3. We conclude that the adaptor protein Kindlin-3 is essential for driving the nonclassical monocyte patrolling response to tumor metastasis in the lung.

Presenter: Messmer, Michelle

Early immune responses to necroptotic death.

Michelle Messmer, Annelise G. Snyder, Michael Y. Gerner, Andrew Oberst

University of Washington

Necroptosis, a programmed form of cell death that results in significant cytokine and chemokine production as well as abundant release of cellular contents to extracellular space, has been demonstrated to more efficiently prime antigen-specific CD8+ T cell responses compared to other forms of cellular death. However, the immunogenic pathway connecting necroptotic cell-derived antigen to T cell priming remains poorly understood. This project seeks to define early immune responders targeted by specific forms of cell death. Using novel activatable pro-death constructs coupled with high-content imaging approaches, we show that necroptotic cell debris rapidly drains to lymph nodes and is taken up by myeloid subsets primarily in the subcapsular sinus, although antigen-bearing myeloid cells are also detectable within the deep T cell zone. Necroptotic antigen-bearing myeloid cells have higher expression of co-stimulatory markers following dying cell injection, concurrent with observed T cell clustering in the lymph node. Better understanding of the mechanisms underlying enhanced T cell priming by necroptotic cells will clarify settings such as in tumor therapy, where specific death induction could be optimally paired with immune interventions.

Presenter: Miller, Corey

Thymic tuft cells promote a medullary microenvironment rich in type 2 cytokines and shape T cell development

Corey Miller, Irina Proekt, Jakob von Moltke, Haiguang Wang, Kristin Rattay, Kristen Lyn Wells, Imran Khan, Bruno Kyewski, Kristin Hogquist, Lars Steinmetz, Richard Locksley, Mark Anderson

University of California, San Francisco

ABSTRACT TEXT NOT AVAILABLE ONLINE

ICOS as a unique source of PI3K signaling in regulatory T cells

Kristen Mittelsteadt, Daniel J. Campbell

University of Washington, Benaroya Research Institute

Foxp3+ regulatory T cells (Treg) are critical for maintaining immune tolerance and preventing inflammatory disease at different tissue sites. Recent work suggests that signaling through the co-stimulatory receptor ICOS is required to control Treg cell abundance and function in vivo in peripheral tissues and sites of inflammation. ICOS signaling primarily results in strong PI3K activation. Though Treg are thought to employ mechanisms to suppress PI3K signaling downstream of the TCR and cytokine receptors compared to conventional T cells (Tconv), mice expressing catalytically inactive PI3K exhibit severe defects in Treg maintenance and suppressive function. Taken together, we hypothesize that ICOS is an important source of PI3K signaling in Treg. Indeed, we find that while Tconv are capable of activating PI3K in response to multiple inputs, stimulation of sorted Treg through ICOS results in potent phosphorylation of AKT downstream of PI3K without any synergistic effect of additional stimuli. Mice lacking ICOS (KO) or carrying a knock-in mutation that specifically abolishes ICOS-dependent PI3K signaling (YF) have reduced Treg frequencies in lymphoid and non-lymphoid tissues. YF and KO Treg have impaired regulation of downstream PI3K targets, including decreased phosphorylation of AKT and S6 and altered expression of the lymphoid homing molecule CD62L. In mixed bone marrow chimeras, YF and KO Treg preferentially repopulate lymphoid organs and exhibit a competitive disadvantage in reconstituting non-lymphoid tissues compared to WT Treg. Furthermore, YF and KO mice are unable to recover from both active and passive transfer experimental autoimmune encephalomyelitis, correlating with lower frequencies of activated Treg at effector sites. Taken together, we suggest an important role for ICOS-mediated PI3K activation in the maintenance and function of Tregs.

Presenter: Mouchess, Maria

Loss of renal DNase1 expression potentiates immune complex deposition and autoimmune lupus nephritis

Maria Mouchess, Eric Suto, Lydia Santell, Sara Chan, Bingbing Dai, Martine Darwish, Dongping He, Susan Haller, Wendy Lam, Chris Bjornson, Jeff Eastham-Anderson, Yanmei Lu, Craig Blanchette, Wyne Lee, Cary Austin, Bob Lazarus, Tangsheng Yi

Genentech, Department of Immunology Discovery

ABSTRACT TEXT NOT AVAILABLE ONLINE

Presenter: Moussion, Christine

STAMP, Skin Tumor Arrays by Micro-Poration

Christine Moussion, Guadalupe Ortiz-Muñoz

Genentech

ABSTRACT TEXT NOT AVAILABLE ONLINE

Presenter: Mullins, Genevieve

Signaling responsiveness driving autoimmune disease pathology and kinetics

Genevieve Mullins, Kristen M. Valentine, Mufadhal Al-Kuhlani, Dan Davini, and Katrina K. Hoyer

Univeristy of California, Merced

IL-2Rα is the high affinity receptor for IL-2, a cytokine important in immune proliferation, activation, and regulation. Mice deficient in IL-2Rα (IL-2Rα-KO) develop systemic autoimmune disease and die from severe anemia between 18-80 days of age. These mice develop kinetically differing autoimmune disease, with approximately a quarter dying by 21 days and half dying after 30 days. This research aims to evaluate the kinetic and phenotypic differences between early- and late-stage autoimmune disease in IL-2Rα-KO mice. To investigate the kinetic differences and the extent of peripheral autoimmune hemolytic anemia and bone marrow failure, we evaluated complete blood counts, antibody binding of RBCs, T cell numbers and activation, and hematopoietic stem cell (HSC) lineage changes. Our data indicate that IL-2Rα-KO mice develop both disease phenotypes largely regardless of disease kinetics, although autoimmune hemolytic anemia is more severe in mice predicted to die early. Upon further exploration, we identified differences in CD4 and CD8 T cell capacity to produce and respond to the cytokines IL-2, IL-7, and IFNγ. We observed differences in expression of these cytokine receptors and in phosphorylation of downstream signaling pathways. We are currently exploring the role of these signaling differences in the disease kinetics and pathologies.

Presenter: Murugan, Dhaarini

C5aR Blockade Increases Intratumoral Clonal T Cell Response in Squamous Carcinomas

Dhaarini Murugan, Wesley Horton, Terry R. Medler, Sushil Kumar, Tiziana Cotechini, Patrick Leyshock, Alexandra M. Forsyth, Justin J. Leitenberger, Molly Kulesz-Martin, Adam Margolin, Zena Werb, and Lisa M. Coussens

Oregon Health and Science University

Activation of complement cascade factors in neoplastic tissues has been paradoxically linked to both cancer progression, and cancer restriction. As a protumoral mediator, activation of terminal complement proteins (C5a) leads to recruitment and activation of leukocytes into "damaged" tissues. To investigate potential T cell-suppressive properties of complement mediators, we have utilized the K14-HPV16 transgenic mouse model of squamous carcinogenesis to evaluate functional mediators of complement activation, and downstream effector programs regulating T cell functionality. Our data have revealed that urokinase (uPA)-positive macrophages regulate C3-independent activation of complement C5a in neoplastic tissue, that in turn mediate suppression of CD8+ T cell cytotoxicity. Therapeutic inhibition of C5a receptor (C5aR1) with a peptide antagonist improved efficacy to paclitaxel chemotherapy associated with CXCR3-dependent CD8+ T cell activation. To investigate the effects of combination therapy on the T cell repertoires infiltrating responding tumors and in peripheral blood, we performed deep sequencing of the complementarity-determining region (CDR) 3 of the T cell receptor (TCR)β chain in matched tumor lysates and peripheral blood mononuclear cells (PBMCs). Combination therapy induced a significant reduction in both circulating and intratumoral TCRβ diversity indicating selective enrichment of specific T cell clonotypes. In addition, clonal homeostasis analyses revealed increased hyperexpanded clones in tumor, and a corresponding increase in the medium frequency clones in PBMCs. Further, combination therapy increased TCRβ clonotype sequence commonality between tumor and PBMCs as identified by pairwise analyses. Together, these data indicate that C5aR1-blockade in combination with paclitaxel chemotherapy facilitates infiltration of antigen-specific T cell clones into tumors and associated with decreased tumor burden and slowed tumor kinetics. In addition, these data highlight the importance of targeting C5aR1-dependent signaling pathways as important immune modulatory pathways in squamous carcinomas for anti-cancer immunotherapy.

Presenter: Naradikian, Martin

Identifying and engineering TCR specificity against mutated self

Martin Naradikian, Leslie Montero, Samantha Hall, Claire Chen, Milad Bahmanof, Luise Sternberg, Jerome Lane, Zeynep Kosaloglu-Yalcin, Manasa Lanka, Aaron Miller, Bejorn Peters, Ezra Cohen, Stephen Schoenberger

La Jolla Institute for Allergy and Immunology

With the recent successes of checkpoint inhibitors and chimeric-antigen receptor (CAR) T cell therapy against various malignancies, immunotherapy has emerged as a new standard of care in the field of oncology. However, these approaches fail to impart specificity against cancer neo-antigens. Indeed, while CD19-specific CAR T cells do eliminate B cell leukemia, they also eradicate normal B cells. Adopting a similar approach for solid tumors will prove difficult because most malignancies do not have lineage-determining surface markers as well restricted as CD19 is to B cells and are typically shared with vital tissues and organs. Thus, there exists a need to engineer specificity against "mutated self". Here, we report a novel methodology for determining immunogenic, mutated-self peptides in multiple cancers using whole exome and transcriptome sequencing of patient-derived tumor biopsies. Importantly, using these peptides, we empirically show that patients harbor neo-antigen specific CD4 and CD8 T cells in both TIL and blood. By sorting cytokine-secreting T cells and performing single-cell sequencing, we have identified TCRs that recognize common driver mutations. Moreover, we validate these TCR constructs by engineering T cells and stimulating them with proper MHC-restricted antigen presenting cells. Lastly, we have established inducible cancer stem cell lines from patients and transplanted them into PDX mouse models. Future studies will address whether engineering sufficient numbers of neo-antigen specific T cells will lead not only to the recognition of established tumors but also control and eradication.

Regulation of Myeloid Immune Cell Activation and the Type I IFN Response by Interleukin Enhancer Binding Factor 3 (ILF3)

Rudy Nazitto, Jarrod S Johnson, Lynn M Amon, Alan Aderem
University of Washington/Center for Infectious Disease Research

Type I interferon (IFN) acts as a potent antiviral weapon. Almost all cells can produce type I IFN, but innate immune cells, such as myeloid dendritic cells (DCs), play key roles in IFN signaling as they are positioned to "sense" pathogens and program adaptive immune responses. It is critical that we understand IFN regulation, since dysregulation occurs in disorders such as autoimmune disease, cancer, and chronic infections. In particular, HIV-1 is known to evade early detection in myeloid DCs, but as the disease progresses, IFN responses become exacerbated and correlate with the degree of pathogenesis. Using systems biology approaches, we have uncovered that Interleukin Enhancer Binding Factor 3 (ILF3) acts as a negative regulator of HIV-driven innate immune responses. ILF3 is known to have a role in regulating stress responses and host mRNA in ribonucleoprotein (RNP) complexes, and our work suggests an additional role in regulating cytoplasmic DNA sensing. Here we find that shRNA knockdown of ILF3 in MDDCs led to increased basal activation and significantly potentiated maturation (CD86, HLA-DR, CD40) and IFN production during HIV-1 infection. Inhibition of HIV-1 reverse transcription completely abrogated maturation and IFN induction, consistent with the role for cytoplasmic DNA as a trigger for innate sensing. Surprisingly, when HIV integration was blocked, cells deficient in ILF3 still exhibited potentiated IFN responses and increased maturation, suggesting ILF3 may contribute to regulation of cytoplasmic cDNA sensing through the established DNA sensor for HIV-1, cGAS. Transcriptomic analysis of ILF3-deficient MDDCs paired with motif scanning of differentially regulated transcripts has given us insight into how ILF3 might regulate signaling thresholds in innate immunity. This work uniquely links host RNA-binding complexes to modulation of innate sensing, antiviral responses, and myeloid maturation.

Presenter: Nguyen, Minh Quan

Characterization of T Follicular Helper Cells and T Follicular Regulatory Cells in HIV-Infected and Non-Infected Patients

Minh Quan Nguyen, Minh Quan T. Nguyen, Rachel S. Resop, Brent Gordon, Marta Epeldegui, Otoniel Martinez-Maza, Christel H. Uittenbogaart

UCLA

Background: The humoral response is important in fighting bacteria and other intracellular pathogens by the production of specific antibodies by B cells. In the germinal center, T follicular helper (gcTFH) cells provide important help to B cell antibody production. gcTFH have been shown to contribute to HIV persistence and act as an HIV reservoir. Yet, the recently discovered germinal center T follicular regulatory (gcTFR) cells, which inhibit the function of T follicular helper cells, express very similar surface markers as gcTFH. FOXP3 is the only marker that distinguishes gcTFR from gcTFH. Thus, it is unknown whether the increase in gcTFH which has been observed in HIV infection is also due an increase in gcTFR and whether gcTFR, like gcTFH can also contribute to HIV persistence. Methods: Using multicolor flow cytometry, peripheral blood mononuclear cells (PBMC) were stained for intracellular TFR markers: FOXP3 and BCL6, in combination with other cell surface markers including CD4, CD3, CD8, CD25, TIGIT, PD1 and ICOS as well as the HIV co-receptor, CCR5. We used available cryopreserved PBMC from HIV-infected and non-infected individuals in the UCLA Multicenter AIDS Cohort Study (MACS), as opposed to the scarcer lymph nodes. Results: In our study, we have characterized and identified the presence of CD3+CXCR5+CD8-BCL6+ peripheral blood T follicular helper (pTFH) and CD3+CXCR5+CD8-FOXP3+ peripheral blood T regulatory cells (pTFR) in PBMC. Our results show that unlike gcTFR, pTFR do not express B cell lymphoma 6 (BCL6), a TFH master regulator. In HIV-infected individuals, the percentage of pTFH is higher. However, the percentage of pTFR is similar in HIV-infected and non-infected individuals. Our results show that there is a statistically significant difference between the levels of ICOS expressed on pTFR from HIV-infected than non-infected individuals. ICOS is expressed on activated T cells and its constitutive expression was shown on regulatory T cells. We noticed constitutive expression of ICOS on pTFR from non-infected individuals and that HIV infection increased its expression on pTFR. The expression of Programmed Cell Death Protein 1 (PD1) is increased on both pTFH and pTFR in HIV-infected individuals. pTFR expressed higher levels of TIGIT than pTFH, but there was no difference between the expression of TIGIT on cells from non-infected and HIV-infected individuals. Both pTFH and pTFR express from 2% to 42% of CCR5, indicating their susceptibility to CCR5-tropic HIV and a potential to contribute to HIV reservoir. Conclusion: In summary, our data show that pTFH and pTFR can be detected in peripheral blood of HIV-infected and non-infected individuals and potentially serve as a representative measure of gcTFH and gcTFR inside the lymph nodes. Although the percentage of pTFR is not elevated in HIV-infected individuals, we do not yet know whether their function is affected or whether they harbor HIV and thereby contribute to HIV persistence. Experiments are planned to address these questions. Further understanding of cells contributing to HIV persistence will be beneficial to the discovery of a functional cure for HIV infection.

BCAP inhibits inflammasome assembly and activation through interactions with Flightless-1

Minjian Ni, Samuel J. Carpentier a, Jeffrey M. Duggan b,c, Richard G. James d,e, Brad T. Cookson a,f, Jessica A. Hamerman b,c, aDepartment of Microbiology, University of Washington, Seattle, WA 98195 bImmunology Program, Benaroya Research Institute, Seattle, WA 98101 cDepartment of Immunology, University of Washington, Seattle, WA 98195 dSeattle Children's Research Institute, Seattle WA 98101 eDepartment of Pediatrics, University of Washington, Seattle, WA 98195 fDepartment of Laboratory Medicine, University of Washington, Seattle, WA 98195

Benaroya Research Institute, Seattle, WA 98101

Inflammasome activation is a key host defense that contributes to clearance of pathogens, yet aberrant inflammasome activity can lead to inflammatory disorders. Despite the importance of keeping inflammasome activation in check, mechanisms that limit inflammasome function remain poorly understood. Here we demonstrate that the signaling adaptor B cell adaptor for PI3-kinase (BCAP) is a novel endogenous inhibitor of NLRP3 and NLRC4 inflammasome activation in macrophages. BCAP-deficient macrophages had increased NLRP3 and NLRC4-induced caspase-1 activation, cell death, and IL-1β release compared to wild-type macrophages. Accordingly, innate immune clearance of a Yersinia pseudotuberculosis mutant requiring caspase-1 for control was enhanced in BCAP-deficient mice. Mechanistically, BCAP delays the recruitment and activation of caspase-1 to the forming inflammasome through its association with the endogenous caspase-1 inhibitor Flightless-1. Thus, we have identified a novel inhibitory pathway that regulates inflammasome activation and innate immune cell function.

Presenter: O'Brien, Carleigh

ICOS signaling suppresses an excessive T cell response in the CNS during chronic T. gondii infection

Carleigh O'Brien, Tajie Harris

University of Virginia

CNS infection with the parasite Toxoplasma gondii results in ongoing T cell recruitment and chronic inflammation in the brain, which is required for control of the parasite. It has also been shown that immunosuppressive cytokine production during this chronic inflammation is important in preventing severe immunopathology associated with this chronic effector response, however, very little is known about the specific mechanisms promoting regulation of long-term inflammatory responses in the CNS. ICOS (inducible T cell costimulator) is expressed on activated T cells and has been shown to both promote inflammatory effector T cell responses in acute infection models, as well contribute to immunoregulation through induction of IL-10. The role of ICOS signaling during the maintenance of long-term immune responses to chronic inflammation still remains poorly understood. We find ICOS expressing T cells in the brain during chronic T. gondii infection, and blocking ICOS signaling at this stage of infection unexpectedly leads to increased effector T cell responses and increased immunopathology in the CNS. This effect appears to be limited to the inflamed CNS, where the increased effector T cell response seen with ICOS-ligand blockade is associated with higher CD25 and pSTAT5 levels, as well as increased proliferation and Bcl-2 expression. Together, these data suggest that ICOS signaling in the context of chronic inflammation negatively regulates IL-2 signaling in effector T cells. Surprisingly, no changes in IL-10 production are seen with ICOS-ligand blockade, suggesting that ICOS signaling promotes regulation of effector T cell responses independent of the effects of IL-10. Indeed, blockade of IL-10R during chronic T. gondii infection does not increase levels of CD25 or Bcl-2 in effector T cells. Overall, the excessive T cell response seen in the absence of ICOS signaling suggests an important role for ICOS-ICOSL costimulation in maintaining local immune suppression in the CNS during chronic infection.

Presenter: O'Sullivan, Timothy

ILC1 confer early host protection at initial sites of viral infection

Timothy O'Sullivan, Weizman, O.E., Adams, N.M., Schuster I., Krishna C., Pritykin, Y., Lau C., Degli-Esposti, M.A., Leslie, C.S., Sun, J.C., and O'Sullivan, T.E.

UCLA

Infection is restrained by the concerted activation of tissue-resident and circulating immune cells. Whether tissue-resident lymphocytes confer early antiviral immunity at local sites of primary infection prior to the initiation of circulating responses is not well understood. Furthermore, the kinetics of initial antiviral responses at sites of infection remain unclear. Here, we show that tissue-resident type 1 innate lymphoid cells (ILC1) serve an essential early role in host immunity through rapid production of interferon (IFN)-? following viral infection. Ablation of Zfp683-dependent liver ILC1 lead to increased viral load in the presence of intact adaptive and innate immune cells critical for mouse cytomegalovirus (MCMV) clearance. Swift production of IL-12 by tissue-resident XCR1+ conventional dendritic cells (cDC1) promoted ILC1 production of IFN-? in a STAT4-dependent manner to limit early viral burden. Thus, ILC1 contribute an essential role in viral immunosurveillance at sites of initial infection in response to local cDC1-derived proinflammatory cytokines.

Rig-I-Like Receptors (RLRs) regulate humoral immunity to West Nile Virus (WNV) infection

Marvin Oketch, Dominik Schenten

Department of Immunobiology, College of Medicine, The University of Arizona

The activation of pattern recognition receptors (PRRs) is a major regulatory checkpoint for the generation of adaptive immunity. Rig-I-like Receptors (RLRs) comprise a PRR family that includes the RNA helicases RIG-I and MDA-5, which recognize microbial RNA in the cytosol. RLR activation induces an anti-viral state in infected cells and leads to the release of proinflammatory cytokines and interferons. RLRs are therefore important mediators of innate immunity to many viral infections. However, the role of RLRs in the regulation of adaptive immunity is still poorly understood. Infection of mice deficient of MAVS, the essential signaling adaptor for RLRs, with West Nile Virus (WNV) results in a defective adaptive immune response. While this finding suggests a role for RLRs in the regulation of adaptive immunity to WNV, it is difficult to interpret due a high WNV viremia in the absence of a MAVS-dependent innate immune response. In order to overcome this caveat, we have infected MAVS-deficient mice with a mutant form of WNV that is unable to assemble newly infectious virions and is therefore limited to a single round of infection. Here we show that MAVS-deficient mice display increased numbers of antigen-specific CD4 T cells as well as an enlarged germinal center (GC) B cell compartment. Importantly, these mice fail to produce an effective neutralizing antibody response to WNV despite normal levels of WNV-specific antibodies. Together, these findings suggest that RLR-dependent signals regulate humoral immunity to WNV.

Presenter: Olson, Rebecca

Innate immune recognition of HIV RNA in latent cells

Rebecca Olson, Sunil Thomas, Michael Gale Jr.

University of Washington

Over 37 million people are currently infected with HIV worldwide. There is no way to completely clear virus from an HIV-infected individual, in large part due to a population of latently infected cells that silently harbor viral DNA while retaining the ability to produce infectious virions upon cell activation. Designing a therapy to eliminate the HIV latent reservoir while sparing uninfected cells remains a major challenge. This task might be accomplished by targeting intracellular innate immune pathways, several of which are known to recognize HIV nucleic acids and induce type 1 interferon in acutely infected cells. However, the establishment of latent infection is associated with innate immune silence and it is not known if HIV products can activate these pathways in latent cells. We are addressing this critical gap in knowledge using the J-Lat CD4+ T cell line model of HIV latent infection. We have found that latent cells have intact type 1 interferon responses that can be triggered by unrelated viruses but not by reactivation of HIV infection alone. We have also identified a unique immune signature of TNFα production and CD59 expression in latent cells transcribing HIV RNA, which is absent in activated cells lacking HIV. This response has not previously been described in the context of HIV latent infection and suggests host recognition of HIV RNA through an NFκB-dependent pathway. TNFα production in latent cells was diminished with the onset of HIV translation, supporting a role for HIV proteins in subverting this response. We are further exploring what innate immune pathways are triggered by HIV RNA upon reactivation of latent infection. Our goal is to define the HIV products that stimulate innate immune signaling and to reveal how HIV proteins regulate these pathways. These studies will provide insight on the host response to HIV infection and will inform new therapies that harness intracellular innate immunity to eradicate the latent reservoir.

Presenter: Osborn, Jossef

Memory CD8+ T cell-mediated protective immunity broadly limits tissue-residency differentiation in the skin during viral infection

Jossef Osborn, Jeffrey C. Nolz

Oregon Health & Science University

Infection of the skin with Vaccinia virus (VacV) generates both tissue-resident memory (TRM) CD8+ T cells and a population of circulating memory CD8+ T cells. Following re-infection, circulating memory CD8+ T cells rapidly enter inflamed skin, where they provide antigen-specific protective immunity. However, whether these circulating protective memory CD8+ T cells impact the local tissue microenvironment or develop into TRM is largely unknown. Here, we show that acute LCMV infection results in substantial circulating CD8+ T cell memory, while remarkably few TRM are seeded into the skin. Infection of LCMV-immune mice with VacV-GP33 resulted in rapid recruitment of circulating memory CD8+ T cells into the skin. These cells were generally CD127+/KLRG1-, expressed cytolytic granzyme, and provided protective immunity prior to the recruitment of re-activated memory CD8+ T cells that proliferated in the draining lymph node. Because we found that naÏve CD8+ T cells require lymph node access to become activated following VacV skin infection, we investigated whether L-selectin (CD62L) was also required for a memory CD8+ T cell response against VacV. In contrast to naÏve cells, memory CD8+ T cells trafficked into the skin and established TRM without requiring CD62L-mediated lymph node priming. Both WT and CD62L-deficient memory CD8+ T cells also produced TRM that expressed less CD69 and CD103 than those generated by previously naÏve CD8+ T cells. Together, these data agree with a model wherein memory CD8+ T cells retain the ability to establish TRM following infection, but reduced viral load and/or local inflammation results in skewed expression of canonical TRM-identifying surface proteins. Our findings suggest that tissue-derived signals in the local microenvironment are essential for circulating memory CD8+ T cells to transition to TRM, which may ultimately have implications for the treatment of T cell-mediated autoimmune conditions and tissue-specific vaccine development.

T Lymphocyte Calcium Dynamics Using a Ratiometric tdTomato-GCaMP6f Transgenic Reporter Mouse

Shivashankar Othy, Tobias X. Dong, Amit Jairaman, Jonathan Skupsky, Angel Zavala, Ian Parker, Joseph L. Dynes, and Michael D. Cahalan

Department of Physiology and Biophysics, University of California Irvine, CA 92697-4561, USA

Calcium is an essential cellular messenger that regulates numerous functions in living organisms. Currently available synthetic calcium indicators such as fura-2 and fluo-4 are unsuitable for long-term studies due to leakage out of cells, and fluorescent protein-based GECI such as GCaMP6 (non-ratiometric) are problematic for calcium imaging in motile cells where fluorescence changes resulting from movement may be indistinguishable from actual changes in calcium levels. Here, we describe development and characterization of "Salsa6f", a fusion of GCaMP6f and tdTomato optimized for cell tracking while monitoring cytosolic calcium. We created a transgenic mouse strain in which Salsa6f is expressed under cell-specific genetic control in Cre-driver strains using the Rosa26-Cre recombinase system, and we have used this system to monitor cytosolic calcium in CD4-Salsa6f T cells. Salsa6f expression is non-perturbing; we saw no effects of Salsa6f expression in T cell surface phenotype, cell proliferation, differentiation, homing, and motility in the lymph node. Salsa6f is distributed uniformly throughout the cytosol; its exclusion from the nucleus provides reliable and selective reporting of cytosolic calcium signaling. We describe single cell calcium signals reported by Salsa6f during T cell receptor activation in naïve T cells, Th17 cells and regulatory T cells, and calcium signals mediated in T cells by an activator of mechanosensitive Piezo1 channels. Transgenic expression of Salsa6f enables ratiometric imaging of calcium signals in complex tissue environments found in vivo. Two-photon imaging of migrating T cells in the steady-state lymph node revealed both cell-wide and localized sub-cellular calcium transients ("sparkles") as cells migrate. Altogether, our results demonstrate the sensitivity, brightness, uniformity of labelling, and ease of detecting calcium signals in moving T cells using Salsa6f.

Presenter: Ou, Pengju

A novel factor regulating cross-presentation in CD8+ dendritic cells

Pengju Ou, Xiaoli Liu, Lifen Weng, Ling Wang, Chaofei Su, Hailiang Wu, Xiaoling Huang, Hai Ni, Cliff Y Yang

Zhongshan School of Medicine, Sun Yat-Sen University

Cross-presentation is an important antigen presentation pathway to prime CD8+ T cells to eliminate pathogens and tumors. Although there is enormous interest in the molecular mechanism of cross-presentation, it is unclear how the cross-presented antigen is trafficked through endosomal compartments. According to our preliminary data, PPT1 (Palmitoyl-protein thioesterase 1) is specifically expressed in CD8 + dendritic cells at a high level, and knockout mice have a greatly enhanced ability to cross-prime CD8+ T cells. Thus, we hypothesize it may play an important role in the cross-presentation ability of CD8+ dendritic cells. Using transgenic mouse models, we have examined the function of this protein in cross-presentation ability of CD8+ dendritic cells under steady state and inflammatory conditions, evaluated the importance of this protein in immune response with infection and analyzed the molecular mechanism of how it controls cross-presentation in CD8+ dendritic cells. This project will not only further our understanding of the molecular mechanism of cross-presentation, it will also facilitate the design of a novel immunotherapy against infectious diseases and cancer by modulating its protein activity.

Presenter: Pinkard, Henry

Dynamic, whole-lymph node imaging and single cell tracking using sample-adaptive two photon microscopy

Henry Pinkard, Adriana Mujal, Hratch Baghdassarian, Daniel Friedman, Ivana Malenica, Adam Fries, Daniel Fletcher, Laura Waller, Matthew Krummel

UCSF, UC Berkeley

Dynamic imaging of lymph nodes using two-photon microscopy has been a major driver of our understanding of the spatiotemporal sequence of cell-cell interactions that drive immune responses. However, such experiments present two major technical challenges, which has limited their applicability to small subvolumes of lymph nodes with several orders of magnitude higher than physiological clonal cell frequencies: 1) The challenge of imaging large volumes of intact, living lymph nodes 2) The challenge of identifying individual cells within such a volume and tracking their movements. In this work, we demonstrate the solutions to each of these problems. Taking inspiration from the technique of standard candles in astronomy, we demonstrate that fluorescent lymphocytes can be used as markers that enable a machine learning algorithm to automatically control excitation laser power in response to the local morphology of the tissue. This enables two-photon imaging to be scaled to the whole organ level. Next, we show an algorithmic pipeline that uses 3D segmentation algorithms to identify potential cells, and interactive machine learning to efficiently label informative subsets of these potential cells. This allows populations of labelled cells to be identified based on their morphology and fluorescent spectra with minimal manual effort. Together these technologies enable a major advance in our ability to visualize immune dynamics over large areas of space in physiologically realistic conditions.

Airway epithelium from asthmatic patients have altered immune responses to rhinovirus infection

Kathryn L Pothoven, Kaitlyn A Barrow, Jason S Debley, Steven F Ziegler

Benaroya Research Institute, Seattle Children's Research Institute

Airway epithelia are the interface between the lungs and the environment. They play important roles in homeostasis of the airways, the immune response to pathogens and in inflammation. Epithelia from asthmatic patients have been shown to be structurally dysfunctional through the loss of differentiated cells, impaired barrier function, and altered cytokine expression including elevated IL-33 and TSLP. Human rhinovirus (HRV) respiratory infection is a common trigger of asthma exacerbation in both children and adults. We hypothesize that the airway epithelial immune response to HRV contributes to subsequent exacerbations in asthma patients. Airway epithelium from asthmatic patients and healthy controls were grown at air liquid interface (ALI) to allow the epithelial cells to fully differentiate as they would in vivo. Once the ALI cultures were fully differentiated, they were either left uninfected or infected with a 0.1 MOI of HRV16 for 48 or 96 hours. RNA was collected and analyzed. Both healthy and asthmatic epithelial cultures expressed elevated CXCL10 transcript compared to uninfected controls at 48 hours, suggesting that ALI cultures had been infected. However, ALIs from asthma patients expressed more CXCL10 compared to ALIs from healthy controls. Baseline expression of the type 2 epithelial cytokines TSLP and IL33 was also elevated in ALIs from asthma patients compared to controls and did not change upon infection at 48 hours, suggesting that asthmatic epithelia may have an elevation in both type 1 and type 2 immune responses to HRV infection. Interestingly, at both 48 and 96 hours the epithelial mesenchymal transition (EMT) marker, vimentin (VIM) was elevated in both HRV infected and uninfected epithelia from asthmatic patients compared to controls, suggesting that EMT may play a role in the pathogenesis of asthma. Additionally, the pro-repair growth factor, amphiregulin (AREG) was decreased in asthmatic epithelia following HRV infection. These data suggest that asthmatic epithelia may have an elevated type 1 and type 2 immune response, and may also have dysfunctional repair mechanisms in response to HRV infection.

Presenter: Powell, Daniel A.

Determining Mechanisms of Protection In a Live Attenuated Coccidiodes Vaccine

Daniel A. Powell, Lisa F. Shubitz, Lourdes Lewis, Christine D. Butkiewicz, Marc J. Orbach, John N. Galgiani, Jeffrey A. Frelinger

University of Arizona

Coccidioidomycosis, caused by the two fungal species Coccidioides immitis and C. posadasii, is an endemic disease in northern Mexico and the southwestern United States. Our goal is to develop an effective vaccine. Previous work in our laboratory has shown that vaccination with a live attenuated mutant Δcps1 can protect mice from a lethal intranasal Coccidioides challenge. This protection is durable, lasting at least six months post vaccination, and effective regardless of the vaccination route (intranasal, subcutaneous, intraperitoneal). Here we dissect the mechanisms of protection using cellular transfers into naĬve mice. C57BL/6J (B6) mice were vaccinated twice subcutaneously with 50,000 spores of Δcps1 2 weeks apart. Ten days after boosting mice were sacrificed and spleens and serum were harvested. Spleen cells for transfer were fractionated using negative selection. Serum or fractionated spleen cells were transferred to naĬve mice intraperitoneally. The following day mice were challenged with a lethal intranasal dose of C. posadasii, strain Silveira, spores. Mice were sacrificed at 14 days post challenge for lung fungal burden quantitation. Transfers of splenocytes, but not serum from vaccinated animals resulted in a significantly reduced fungal burden compared to unvaccinated cellular transfers. Flow cytometric analysis indicated that vaccination produces antigen specific CD4+ IFN-γ+ T cells. Further cellular fractionation showed that splenocytes depleted of T cells had no protective effect; conversely, transfer of CD4+ cells showed a similar effect to whole spleen transfers. CD4+, but not CD8+, cellular transfer can reduce fungal burdens after lethal C. posadasii challenge. The fact that transfer of serum had no effect indicates the humoral immune response was not responsible for the protective effect imparted by Δcps1 vaccination. Further work is being carried out to determine the direct effect of the CD4+ cells on Coccidioides challenge.

Presenter: Radu, Caius

Nucleotide metabolism reprogramming by type I interferons in pancreatic cancer

Caius Radu, Evan R. Abt, Amanda Dann, Thuc M. Le, Joseph R. Capri, Timothy R. Donahue
University of California Los Angeles

Pancreatic ductal adenocarcinoma (PDAC) is the third-leading cause of cancer-related deaths in the United States with an overall survival of less than one year. An improved knowledge of PDAC biology, to uncover vulnerabilities specific to cancer cells, is needed to develop more effective therapeutic options. We are investigating the intersection between three aspects of PDAC biology that can, ultimately, be developed for therapeutics: (i) cytokine signaling, with a particular focus on the metabolic effects of interferons (IFNs), which are present in the highly inflamed and dense PDAC stromal microenvironment; (ii) nucleotide metabolism, a network of tightly regulated biochemical pathways that produce deoxyribonucleotide triphosphates (dNTPs) which are required for DNA replication; and (iii) the replication stress response pathway, an intracellular signaling mechanism which is activated by perturbations in DNA replication, and has been recently shown to govern key aspects of nucleotide metabolism. Using an integrated metabolic, transcriptomic, and proteomic analytical platform we show that IFN signaling in PDAC cells induces a switch in nucleotide metabolism from a biosynthetic to a predominantly dNTP catabolic phenotype. This switch appears to be mediated by dNTP phosphohydrolysis catalyzed by the Sterile Alpha Motif and Histidine/aspartic acid Domain-containing protein (SAMHD1). We also demonstrate a role for the replication stress response kinase Ataxia Telangiectasia and Rad3-related protein (ATR) in regulating dNTP levels in PDAC cells exposed to IFN. Furthermore, we have demonstrated that ATR activity is an actionable co-dependency of IFN-exposed cells and that pharmacological inhibition of ATR triggers apoptosis in PDAC cells exposed to IFN. Collectively, these studies increase our understanding of the interplay between IFN signaling, replication stress signal transduction networks, and nucleotide metabolism in PDAC, and uncovered critical vulnerabilities to be exploited by new therapeutic approaches against this extremely aggressive and difficult to treat malignancy.

Presenter: Roberts, Edward

Migratory Dendritic Cells Activated in the Tumor Microenvironment Seed Lymph Node Resident Populations with Tumor Antigens Using Distinct Pathways

Edward Roberts, Roberts EW*, Ruhland MK*, Mujal AM, Bepplar C, Marchuk K, Cai E, Binnewies M, Fries A, Krummel MF

UCSF

Lymph node resident dendritic cells have important roles in supporting effective CD8+ T-cells priming; however, how these DC acquire antigen is controversial. The mechanism by which antigen is acquired has important consequences for the fate of that antigen influencing the efficiency of and context within it is presented to CD8+ T cells. Here we demonstrate that tumor derived antigen is actively brought into the draining lymph node and is seeded into resident DC by direct cell contact dependent hand off. This mechanism of passing does not occur in the healthy tissue as, despite migratory dendritic cells loading with the same antigen when it was present as a normal tissue antigen, no passing of antigen was observed to resident DC. Although no passing was observed in this tolerogenic context, it could be induced by damaging tissue with low doses of irradiation. In the tumor context antigen was passed by different mechanisms to different resident dendritic cell populations within the lymph node with inhibitor studies suggesting that CD8a+ dendritic cells specifically acquiring antigen through LC3 associated phagocytosis. Administration of melanin, an inhibitor of LC3 associated phagocytosis, in vivo could thus block accumulation of tumor antigen in the resident CD8a population. We now seek to understand the impact of this pathway in priming anti-tumor immune responses.

Presenter: Rosenthal, Rebecca

Age Associated B Cells Represent a Tissue Resident Memory B Cell Subset

Rebecca Rosenthal, Arpita Myles (Co-First Author), Elinor Willis, Scott Hensley, Mike Cancro
University of Pennsylvania

Age Associated B Cells (ABCs) are a newly described memory B cell subset characterized by TBET expression. The ABC subset enlarges with age and is associated with both viral immunity and humoral autoimmunity. Our lab recently showed that a triad of signaling events - TLR engagement, TFH cytokine milieu, and BCR signaling - act in concert to govern ABC development. Furthermore, we have shown that Tbet+ B cells use a diverse array of VH and VL genes, are somatically mutated, and when induced by viral infection, persist indefinitely. Based on these and other findings, our current working model is that Tbet+ B cells are a memory B cell subset that arises during germinal center reactions driven by nucleic acid containing antigens. Surprisingly, following PR8 influenza infection ABCs are largely restricted to the spleen and are entirely absent from the draining lymph nodes. This intriguing observation raised the question of whether ABCs are a splenic tissue resident B cell subset or if they migrate under basal conditions. To address this question, our lab undertook a parabiosis based approach to investigate ABC residency. We parabiosed B6 mice 30+ days post PR8 infection with naïve B6.SJL congenic partners. We observed that while PR8 specific TBET- B cells achieve significant mixing in parabiotic pairs, TBET+ PR8 specific B cells remain in the infected partner and are not seen in the naïve conjoined mouse, showing that the TBET+ ABC population is tissue resident in the spleen.

Presenter: Ruscher, Roland

CD8aa+ Intestinal Intraepithelial Lymphocytes (IEL) Arise from Two Major Thymic Precursor Subsets

Roland Ruscher, Rebecca L. Kummer, You Yeong Lee, Stephen C. Jameson, Kristin A. Hogquist
University of Minnesota Center for Immunology

TCRaß+ CD8aa+ intestinal intraepithelial lymphocytes (CD8aa IEL) descend from thymic precursors. To better define this IEL precursor (IELp) population, we analyzed their maturation, localization, and emigration. Using rigorous lineage exclusion criteria, we defined two precursors among DN TCRß+ thymocytes: a nascent PD-1+ population and a T-bet+ population that accumulates with age. Both gave rise to intestinal CD8aa IEL upon adoptive transfer. PD-1+ cells contained more strongly self-reactive clones, localized to the cortex, and were dominant in S1PR1-dependent thymic egress. Gut homing a4ß7 was expressed by these IELp already at a thymic stage. The T-bet+ IELp population localized to the medulla and expressed NK1.1, CXCR3 and CD103, as well as IFN? reporter protein. The two populations further differed in TCR Va usage and MHC- restriction: While PD-1+ IELp were classical MHC class I restricted, the T-bet+ IELp were dependent on non-classical MHC class I. These data provide an important foundation for understanding the biology of this abundant population of barrier surface T cells.

Leveraging Chemerin and its Receptors in Immune Defense Against Breast and Prostate Cancer

Nicole Salazar, Nicole Salazar, Yayue Zheng, Leona Nease, Jesse Rosalez, Russel Pachynski, Eugene Butcher, Brian Zabel

Stanford School of Medicine

Background: Breast and prostate cancer kill approximately 67,000 people every year in the US. Our lab has previously shown the significant growth inhibiting effect of chemerin in melanoma. ChemerinÂ is a secreted protein with a complex but well-established role in immune function capable of attracting anti-tumor immune cells such as macrophages and natural killer cells. Hypothesis: We asked if chemerin and its stroma-expressed presenting receptor CCRL2 are downregulated by tumors as part of a conserved tumor immune evasion strategy, and if chemerin and CCRL2 enhance anti-tumor immunity in mouse models of breast (BrCa) and prostate cancer (PrCa). Methods: We generated chemerin over-expressing cancer cell lines and chemerin and receptor knock-out mice to 1) determine whether tumor-expressed chemerin inhibits the establishment or growth of tumors and 2) define the independent roles of chemerin and CCRL2 in tumor suppression. We evaluated the in vivo anti-tumor effects of chemerin and CCRL2 in transplantable orthotopic EMT6 and 4T1.2 mammary carcinoma models, and a transplantable ectopic TRAMP-C2 prostate adenocarcinoma model. At the conclusion of the study, we resected the tumors and assessed the composition of the tumor infiltrating leukocyte population by flow cytometry. To extend the translation relevance we assessed gene expression levels of chemerin and CCRL2 in human tumor vs normal tissues. Results: While over-expression of chemerin did not affect the growth rate of mouse BrCa or PrCa cell lines in vitro, when implanted in vivo chemerin significantly suppressed tumor growth. The results were most striking in ectopic PrCa, where no mice developed tumors. In BrCa models, the effect was significant yet less-pronounced. When the TIL populations were plotted as a mean Log2 frequency ratio (chemerin vs. control tumors), there was an increase in anti-tumor leukocytes (NK, CD8+ T cells, total T cells, and DC) and a decrease in immune suppressive, pro-tumor myeloid-derived suppressor cells (MDSC, CD45+CD11b+Gr1+). To ask if host-expressed CCRL2 impacted tumor growth, PrCa TRAMP-C2 cells (which do not express chemerin or CCRL2) were implanted s.c. into WT or CCRL2 KO mice. CCRL2 KO mice developed significantly fewer and smaller tumors than their WT counterparts. This led us to hypothesize that 1) CCRL2 expressed by non-neoplastic stroma normally serves to sequester chemerin away from growing tumors, and 2) tumors down-regulate CCRL2 to limit intra-tumor chemerin accumulation and evade immune surveillance. Indeed, using publicly available human gene expression data, chemerin and CCRL2 were downregulated in many human cancers, and this downregulation correlated with poor survival outcomes, and is consistent with tumor immune evasion. Conclusions: These results demonstrate that chemerin and its receptors can be leveraged to disrupt the growth of breast and prostate tumors, and shift the tumor microenvironment to favor anti-tumor immune defense. These studies may lead to novel chemerin-dependent approaches to engage host immune defenses to slow or reverse cancer progression.

Presenter: Saligrama, Naresha

From T Cell Receptor to antigen, systems approach to discovering T cell antigen(s) in Multiple sclerosis and Experimental autoimmune encephalomyelitis

Naresha Saligrama, Naresha Saligrama, Fan Zhao, William Stuart Serratelli, Ricardo Fernandes, David M Louis, Xuhuai Ji, Vamsee Aditya Mallajosyula, Murad R Mamedov, Arnold S Han, Yueh-hsiu Chien, Christopher K Garcia, Jorge Oksenberg, and Mark M Davis

Stanford University

ABSTRACT TEXT NOT AVAILABLE ONLINE

Enhancer connectome in primary human T cells reveals target genes of autoimmune disease-associated DNA elements

Ansuman Satpathy, Maxwell Mumbach, Evan Boyle, Chao Dai, William Greenleaf, Howard Chang

Stanford University

The challenge of linking intergenic mutations to target genes has limited molecular understanding of human diseases. Here we show that H3K27ac HiChIP generates high-resolution contact maps of active enhancers and target genes in primary human T cell subtypes. Differentiation of naive T cells into T helper 17 cells or regulatory T cells creates subtype-specific enhancer-promoter interactions, specifically at regions of shared DNA accessibility. These data provide a principled means of assigning molecular functions to autoimmune disease risk variants, linking hundreds of noncoding variants to putative gene targets. Target genes identified with HiChIP are further supported by CRISPR interference and activation at linked enhancers, by the presence of expression quantitative trait loci, and by allele-specific enhancer loops in patient-derived primary cells. The majority of disease-associated enhancers contact genes beyond the nearest gene in the linear genome, leading to a fourfold increase in the number of potential target genes for autoimmune disease.

Presenter: Scott, Tia

Role of Ca2+, p42/44 MAPK, and NF-kB signaling in regulating activation-induced chromatin decondensation in T cells

Tia Scott, Jen Funsten, Sarah Eikenbusch, Molly Shields, and Jason S. Rawlings

Furman University

A proper immune response is hallmarked by the activation and clonal proliferation of antigen specific T cells. Antigen presentation to the T cell receptor (TCR) leads to the production of two signaling molecules, IP3 and DAG. While IP3 induces calcium flux, DAG activates Protein Kinase C (PKC). The activation of PKC leads to the activation of two signaling pathways: p42/44 MAP Kinase (MAPK), and NFkB. The activation of these pathways results in the decondensation of chromatin which is required for the subsequent clonal proliferation of activated T cells in response to IL-2. Previous studies have shown that an influx of calcium is sufficient for inducing chromatin decondensation. In this study, we show that calcium is also required for PKC-mediated chromatin decondensation. Furthermore, an influx of calcium is required for gene expression in response to IL-2 stimulation. In comparison, we concluded that while MAPK is not required for chromatin decondensation, it is required for competence to respond to IL-2. Lastly, our data suggest that NFkB is required for proper chromatin decondensation and also contributes to the competence to respond to IL-2. Collectively, these data are suggestive that calcium and PKC signaling cooperate in the processes of chromatin decondensation and subsequent competence to respond to IL-2 during T cell activation.

Antigen transfer in immune homeostasis

Nina K Serwas, Matthew F Krummel

UCSF, Department of Pathology, San Francisco, CA

The ability to tolerate an evolving definition of 'self' is crucial for every living being, however, the way in which 'self' is tolerated as we develop is barely understood. Extracellular vesicles (EVs) can promote tolerance in cancer, and antigen-presenting cells (APCs) can transport EVs to lymph nodes to generate immune responses. As tumors rarely invent pathways but rather hijack available mechanisms, we hypothesize that the immune system uses the flow of EVs from 'true-self' to determine the evolving 'self'. Thus, the role and mechanism of EVs in disseminating 'self'-material deserves considerable attention. Here, we aimed to analyze the first contact of peripheral cells with immune cells. To this end, we co-cultured cancer or primary cells expressing the stable GFP-derivate ZsGreen with bone marrow-derived macrophages. Strikingly, macrophages acquired fluorescent antigen from both, cancer and primary cells which suggested the transfer of self-antigens. We then visualized antigen transfer with high resolution lattice light sheet fluorescent microscopy. Unexpectedly, we observed that, besides large chunks of material, numerous small particles with sizes below 1um were transferred to the macrophages within two hours of co-culture. The transfer mechanism involves close membrane contact of both cells whereas the antigen of the donating cell drips of from protrusion into the macrophages. Future work will address the mechanisms involved in the antigen transfer and relate this process to tolerance maintenance in the immune system.

Presenter: Snyder, Annelise G.

Induction of necroptotic cell death within the tumor microenvironment confers tumor control

Annelise G. Snyder, Michelle N. Messmer, Nicholas W. Hubbard, Susana L. Orozco, Brian P. Daniels, Kristy Chiang, Andrew Oberst

University of Washington, Department of Immunology (Seattle, WA, USA)

Cytotoxic therapies such as irradiation or chemotherapy can promote beneficial anti-tumor immune responses dependent on tumor cell death. However, the specific mechanisms underlying how distinct lytic cell death programs differentially instruct anti-tumor immunity have not been thoroughly investigated. It has been proposed that pro-inflammatory forms of tumor cell death increase the availability of tumor antigen, in addition to providing a burst of inflammatory signals within the typically immunosuppressive tumor microenvironment (TME). Work in our lab and others has shown that a RIPK3-dependent lytic cell death program, termed "necroptosis", stimulates stronger inflammatory immune responses compared to apoptosis; we therefore hypothesized that necroptosis could induce anti-tumor responses within the TME. To test this, we created cell lines in which specific cell death programs can be triggered using bioavailable drug ligands. Using these "Pure Death" systems in syngeneic flank tumor models, we show that introduction of necroptotic cells to the TME leads to CD8-dependent tumor control that is associated with increased tumor antigen loading among tumor-associated antigen-presenting cells, as well as a favorable CD8:Treg ratio. Furthermore, inducing necroptosis within a primary tumor results in the control of distal, untreated tumors, indicating that this therapy confers a systemic response. Further elucidation of the cell subsets and signaling axes responsible for these observations will yield novel insights concerning the contribution of specific tumor cell death programs in promoting anti-tumor immunity.

Presenter: Soysa , Dilini Radika

Kupffer cells resist irradiation through cell cycle arrest

Dilini Radika Soysa , Sarah Margit, Sebastien Yuen, Ian N. Crispe
University of Washington

ABSTRACT TEXT NOT AVAILABLE ONLINE

Presenter: Spath, Sabine

Dysregulation of the cytokine GM-CSF induces spontaneous phagocyte invasion and immunopathology in the CNS

Sabine Spath, Juliana Komuczki, Pawel Pelczar, Bettina Schreiner, Burkhard Becher

Benaroya Research Institute, Seattle, USA and Experimental Immunology, Zurich, Switzerland

Chronic inflammatory diseases are influenced by dysregulation of cytokines. Among them, granulocyte macrophage colony stimulating factor (GM-CSF) is crucial for the pathogenic function of T cells in preclinical models of autoimmunity. To study the impact of dysregulated GM-CSF expression in vivo, we generated a transgenic mouse line allowing the induction of GM-CSF expression in mature, peripheral helper T (Th) cells. Antigen-independent GM-CSF release led to the invasion of inflammatory myeloid cells into the central nervous system (CNS), which was accompanied by the spontaneous development of severe neurological deficits. CNS-invading phagocytes produced reactive oxygen species and exhibited a distinct genetic signature compared to myeloid cells invading other organs. We propose that the CNS is particularly vulnerable to the attack of monocyte-derived phagocytes and that the effector functions of GM-CSF-expanded myeloid cells are in turn guided by the tissue microenvironment.

Presenter: Srivastava, Shivani

Immunogenic chemotherapy markedly enhances the efficacy of ROR1 CAR T cells in an autochthonous model of lung adenocarcinoma

Shivani Srivastava, Sushma Yechan-Gunja, Megha Sarvothama, Stanley R. Riddell

Fred Hutchinson Cancer Research Center

Immunotherapy using chimeric antigen receptor (CAR)-modified T cells has impressive efficacy in hematological malignancies but has been less effective in solid tumors such as lung cancer, which is the leading cause of cancer mortality worldwide. Prior studies of CAR-T cells for solid tumors relied primarily on transplantable and xenogeneic models, which do not replicate the complex tumor microenvironment of human disease. To develop a CAR-T cell therapy model for lung cancer, we adapted the KrasLSL-G12D/+p53flox/flox (KP) mouse model to express the tumor-associated antigen ROR1, which is highly expressed in human lung adenocarcinoma but absent from vital adult tissues. We infected KP mice with Cre-expressing lentivirus to induce deletion of p53 and activation of oncogenic KrasG12D in lung epithelia, mimicking the two most common mutations in non-small cell lung cancer. The lentivirus also encoded ROR1, resulting in expression of ROR1 in nascent tumors. This model replicates initiation and progression of human lung adenocarcinoma, including infiltration by myeloid cells and CD4+ Tregs. Adoptive transfer of ROR1 CAR-T cells into mice bearing established lung tumors modestly controlled tumor growth for the first 6 weeks of treatment but did not improve survival. While CAR-T cells were present in some tumors at 5-fold higher number relative to control T cells, they only infiltrated and induced regression of a small fraction of tumors. Bioluminescence imaging of luciferase-labeled CAR-T cells revealed that most CAR-T cells resided in spleen and lymph nodes rather than the lung, were PD-1-, and most likely ignorant of tumor antigen. The inability of most CAR-T cells to infiltrate lung tumors may be due to the minimal production of T cell-attracting chemokines like CXCL9 and CXCL10 and the fact that a large fraction of circulating CAR-T cells in vivo showed a CD62L-KLRG1+ phenotype that has been associated with poorer ability to traffic to non-lymphoid tissues. Consistent with the impairment of tumor infiltration by CAR-T cells, combination therapy with anti-PD-1 did not enhance antitumor activity despite the expression of PD-1 on CAR-T cells and PD-L1 on tumor-associated myeloid cells. We next tested whether combination therapy of anti-PD-L1 with oxaliplatin and cyclophosphamide (Ox/Cy), which induces immunogenic cell death and may locally induce inflammation, could improve CAR-T cell recruitment to tumors. RNAseq analysis of tumors demonstrated that Ox/Cy upregulated genes involved in immune cell chemotaxis, including Cxcl9, Cxcl10, and Sele. Additionally, a larger fraction of circulating CAR-T cells were CD62L+CD44hi and capable of binding E/P-selectin, enabling extravasation into inflamed tissue, after Ox/Cy treatment. Consequently, Ox/Cy pre-treatment significantly improved CAR-T cell infiltration and induced dramatic regression of a larger fraction of tumors, but did not enhance the activity of control T cells. These data indicate that immunogenic chemotherapy can enhance CAR T cell activity against solid tumors, suggesting this model will be useful to identify combinations for clinical translation.

Communicating Viral Immunity from the Inside Out - How sensing through RIG-I like Receptors leads to Macrophage polarization and Viral control in West Nile Virus infection

Amy Stone, Courtney Wilkins, Richard Green, Michael J Gale, Jr.

University of Washington

RIG-I-Like Receptors (RLRs) include RIG-I, MDA5, and LGP2. RLRs are vital pathogen recognition receptors in the defense against RNA viruses. West Nile Virus (WNV) is an emerging, neurotropic flavivirus that infects the central nervous system to cause disease and death. RIG-I and MDA5 work in tandem to initiate innate immune responses against WNV infection, though the role of LGP2 in this process is not well defined. To gain a deeper understanding of the contributions of each RLR in the innate immune response and immunity to WNV, we conducted a systems biology approach to assess the role of each RLR in immune programming. We conducted genome-wide RNAseq and bioinformatics analysis of WNV infection in bone marrow derived macrophages from wild type mice and from specific RLR-deficient mice. Here we show that the RLRs drive distinct immune gene activation and polarization of the immune response. In our data, the RLR-dependent, WNV-induced immune response polarization overshadows the classical drivers of viral innate immune responses, interferon I (IFN) and IFN-stimulated genes, thus underscoring the importance of innate immune activation for channeling the adaptive immune system into specific effector pathways. Characterization of the genes expressed demonstrated a strong induction of a pro-inflammatory macrophage signature and a suppression of a wound healing macrophage signature in a functional anti-viral response that was lost upon removal of RIG-I and MDA5. These analyses reveal that distinct RLR-driven transcriptional programs lead to differential functional output in immune response initiation against WNV infection. Our study demonstrates a clear, defined role for RIG-I, MDA5, and LGP2 in immune programming against virus infection. Supported by NIH/NIAID 1R01AI104002-01A1 (Gale), T32 AI007509 Diseases of Public Importance Fellowship and F32 AI124520-01 NRSA Individual Postdoctoral Fellowship (Stone).

Presenter: Sun, Lijuan

Re-polarizing tumor-derived macrophages to achieve immune control of breast cancer

Lijuan Sun, Ana Santos Almeida, Tim Kees, Xue-Yan He, Jean Albrengues, David Ng, Sylvia Adams, Mikala Egeblad

Cold Spring Harbor Laboratory

Bone marrow-derived macrophages can be activated in culture to become tumoricidal macrophages using interferon-γ (IFN-γ) and toll like receptor (TLR) agonists. Nevertheless, in hormone receptor (HR)-positive breast cancer, macrophage infiltration correlates with poor prognosis, in part because these macrophages suppress T cell activation. To test whether tumor-associated macrophages can be activated or reprogrammed to become tumoricidal, we isolated macrophages from the bone marrow or primary tumors of genetically engineered MMTV-PyMT mice (a mouse model of luminal B breast cancer), and applied toll-like receptor (TLR) agonists and IFNγ to these macrophages in culture. We found that macrophages activated with IFNγ and either lipopolysaccharide (LPS) or monophosphoryl Lipid A (MPLA) - two TLR4 agonists - killed about 90% of cancer cells in

Presenter: Syrett, Camille

Novel Mechanism for YY1-mediated Control of Dynamic X-chromosome Inactivation Maintenance in Female B Lymphocytes

Camille Syrett, Camille M Syrett, Arpita Myles, Anna Martin, Michael Cancro, Montserrat C Anguera

University of Pennsylvania

The X chromosome is enriched for genes with important functions in immunity. XX females are also more prone to autoimmune disorders such as systemic lupus erythematosus (SLE) where X-linked immune-related genes are overexpressed in lymphocytes. The mechanism underlying this sex bias is unclear, and hypotheses suggest an important role for X-chromosome dosage. Interestingly, female lymphocytes, unlike other somatic cells, uniquely regulate X-chromosome inactivation (XCI), where the inactive X chromosome (Xi) lacks canonical features including heterochromatin modifications and Xist RNA localization. Epigenetic profiling of this unusual Xi reveals that Xist RNA is lost from the Xi in pro-B cells, and that heterochromatic modifications are gradually lost from the Xi during B cell development. Xist RNA and Xi-heterochromatin return back to the Xi upon mature B cell activation in a YY1-dependent fashion. We show that the method of B cell stimulation, involving the B cell receptor or toll-like receptors, influences the timing and patterns of Xist RNA localization to the Xi. To determine if deletion of Xist RNA in lymphocytes causes reactivation of X-linked immune genes, we conditionally deleted Xist in female murine B cells (mb1CRE XistCKO/+). Remarkably, female XistCKO/+ animals develop serum ds-DNA autoantibodies at similar levels to classical lupus animal models, as well as proteinuria and glomerulonephritis. We also observed alterations in mature B cell subsets, as XistCKO/+ animals have elevated numbers of activated, memory, germinal center, and Tbet+ B cells, compared to age-matched controls. We propose that Xist RNA is essential for maintaining the dosage of X-linked immune genes in female B cells, and that perturbations to XCI maintenance contribute to autoimmunity.

Presenter: Tang, Xin-Zi

CX3CR1+ Bronchus-Associated Macrophages are Positioned for Surveillance of the Airways

Xin-Zi Tang, Christopher Allen

UCSF

ABSTRACT TEXT NOT AVAILABLE ONLINE

Presenter: Truckenbrod, Emily

Features of CD8+ T cell tolerance to the self-antigen Trp2

Emily Truckenbrod, Kristin Renkema, Stephen Jameson

Center for Immunology, University of Minnesota

Immunological tolerance to self is critical for avoiding autoimmune pathology, but the nature and enforcement of this tolerance are incompletely understood. Defining these mechanisms is critical for effectively treating autoimmune disease and for safely targeting tumor-associated self-antigens. We are studying the CD8+ T cell response to the melanocyte/melanoma-associated enzyme tyrosinase-related protein 2 (Trp2) in wild-type (WT) C57BL/6 and Trp2-deficient mice to clarify the characteristics of self-tolerance. Deletion of self-reactive T cells during development is a known tolerance mechanism, but we find a relatively large number of Trp2-specific CD8+ T cells in the lymphoid tissues of both WT and Trp2 knock-out (Trp2 KO) mice. Mixed bone marrow chimeras established in these mice using donor cells from Trp2-specific T cell receptor transgenics confirmed that clonal deletion plays a negligible role. We detected a slightly higher avidity for Trp2 in Trp2 KO CD8+ T cells relative to WT cells as measured by Trp2 tetramer median fluorescence intensity. No consistent differences in the expression of activation (CD69, CD44, CD122) or anergy markers (PD-1, CTLA-4, LAG3, 2B4) were identified between Trp2 KO and WT Trp2-specific CD8+ T cells. Despite these relatively modest differences in the Trp2-specific populations from preimmune WT and Trp2 KO mice, we observed striking differences in the performance of Trp2-specific cells from these mice when challenged with Trp2 in an immunogenic context (recombinant Listeria monocytogenes expressing Trp2 or TriVax immunization with Trp2 peptide, poly(I:C), and agonist anti-CD40 antibody). WT mice were poorly responsive to Trp2, while we were able to elicit a functional response to this antigen in Trp2 KO mice. Trp2 KO mice produced a significantly larger number and proportion of Trp2-specific CD8+ T cells at effector and memory time points. Following Listeria infection, the avidity of responding Trp2 KO cells was significantly higher, and cytokine production in response to ex vivo Trp2 stimulation was greater in cells from Trp2 KO mice. Transfers of bulk polyclonal CD8+ T cells suggest a cell-intrinsic tolerance mechanism, as cells from Trp2 KO donors mount a response to Trp2 in both KO and WT recipients whereas cells from WT mice do not respond in either KO or WT recipients. Ongoing work includes assessment of differences in gene expression between tolerant (WT Trp2-specific cells) and non-tolerant cells to clarify the profile of self-tolerant CD8+ T cells and more precisely delineate the intrinsic mechanism restraining tolerant cells in this model. To date, the majority of our work has focused on the Trp2 (180-188) epitope shared between C57BL/6 mice and humans (HLA-A*0201-restricted). However, we have identified similar populations of CD8+ T cells specific for an alternative Trp2 epitope and an epitope from another melanocyte-associated enzyme, tyrosinase-related protein 1. CD8+ T cells specific for these epitopes also escape thymic deletion and the majority have a naïve, not overly anergic phenotype in preimmune WT mice. Our studies of polyclonal CD8+ T cells responding to the same antigen as self vs. foreign represent a physiologically relevant approach likely to reveal insights generalizable to other melanocyte antigens and applicable to recently described non-deletional CD8+ T cell anergy in humans.

Presenter: Uhrlaub, Jennifer

Cell-specific inhibition of SMAD2/3 restores lymph node cellularity and germinal center function in aged mice responding to acute chikungunya virus (CHIKV) infection.

Jennifer Uhrlaub, Jennifer L. Uhrlaub, Yu-Sang Sabrina Yang, Darrell J. Irvine, Janko Nikolich-Å½ugich

University of Arizona

Targeted delivery of small-molecule inhibitors increases drug efficacy by focusing its action upon specific cell-types without global disruption of signaling pathways. TGFβ is a pleotropic cytokine involved in many cellular processes, including specific regulatory functions during anti-viral immune responses. Previously, we have shown that in aged mice, over-production of TGFβ correlates with decreased immune function. TGFβ neutralization during acute chikungunya virus (CHIKV) infection decreases acute- and chronic- disease severity and improves neutralizing antibody titers. Herein, we selectively interrupted TGFβ signaling during acute CHIKV infection via the delivery of lymph node (LN)-targeting nanoparticles coupled to a small-molecule inhibitor of SMAD2/3 phosphorylation. We show that TGFβ signaling is responsible for the poor germinal center reaction and suboptimal antibody production measured in aged mice. Moreover, we show that LN-targeted inhibition of SMAD phosphorylation during the initiation of a viral immune response can restore - to the levels measured in adult mice - the cellular content of the draining lymph node and the germinal center reaction resulting in improved antibody responses.

Presenter: Vasudevan, Sreelakshmi

Masscytometry Based Analysis of Human Lung Macrophages

Sreelakshmi Vasudevan, Joshua Vasquez,Wenxuan Chen,Brandon Aguilar Rodriguez,Siyang Zeng,Erene Niemi,Mehrdad Arjomandi

University of California, San Francisco

ABSTRACT TEXT NOT AVAILABLE ONLINE

ILC2 activation by leukotrienes: NFAT joins the team

Jakob von Moltke, Claire E O'Leary, Nora A Barrett, Yoshihide Kanaoka, K. Frank Austen, Richard M Locksley

University of Washington

The initiation and maintenance of type 2 (allergic) immune responses in the lung requires production of the type 2 cytokines interleukin (IL)-4, IL-5, IL-13, and IL-9, which together drive eosinophilia and other hallmarks of type 2 inflammation. Group 2 innate lymphoid cells (ILC2s) and type 2 helper T cells (Th2) are the primary source of these cytokines. In Th2s, T cell receptor (TCR) signaling activates the transcription factors NFAT, AP-1, and NFκB, which cooperate to induce type 2 cytokines. ILC2s lack a TCR and respond instead to locally produced cytokines such as IL-33. While IL-33 induces AP-1 and NFκB, NFAT signaling has not been described in ILC2s and it remains unclear how maximal cytokine production is achieved in vivo. Here, we report the non-redundant NFAT-dependent role of lipid-derived leukotrienes in the activation of lung ILC2s. Using cytokine reporter and leukotriene-deficient mice in innate and adaptive models of type 2 airway inflammation, we find that while loss of leukotriene B4 or cysteinyl leukotriene signaling alone has modest effects, deletion of both pathways markedly diminishes ILC2 activation and eosinophilia. Type 2 responses are similarly attenuated in IL-33- and leukotriene- deficient mice, and optimal ILC2 activation reflects potent synergy between these pathways. We hypothesize that ILC2s integrate multiple signals as a substitute for TCR signaling. These findings therefore expand our understanding of ILC2 regulation and may have important implications for the treatment of airways disease.

Presenter: Wagar, Lisa

Human tonsil cultures as a model to study adaptive immune responses in vitro

Lisa Wagar, Ameen Salahudeen, Christian Constantz, Katherine Jackson, Michael Lyons, Peter Kim, Calvin Kuo, Scott Boyd, Mark Davis

Stanford University

The human immune system is constantly confronted by a diversity of challenges that require complex and multi-functional responses, encompassing a mixture of immune and non-immune cells types that work in concert within lymphoid structures. Despite its critical importance, our knowledge of how human immune responses are generated has been hindered by a lack of robust in vitro culture methods that reproduce these in vivo interactions. Therefore, we have created an organoid system from human tonsils that recapitulates both heterotypic immune and stromal components and enables in vitro exploration of antigen-specific cellular and humoral responses. The live attenuated influenza vaccine (LAIV) was used as a model antigen to characterize the properties of the culture system. The tonsil cultures have the ability to self-organize and cellular aggregates are enriched for responding cells (such as plasmablasts and T follicular helper cells) after LAIV exposure. We have shown that B cell differentiation and antibody production is strongly dependent on the presence of antigen presenting cells, but not pre-existing memory or germinal center B cell subsets. These organoids can successfully mount a robust humoral response to LAIV and generate microgram quantities of influenza-specific antibodies per culture. Heavy chain BCR repertoire sequencing has revealed a proportion of plasmablasts and germinal center B cells in LAIV-stimulated cultures have CDR3 motifs that are known to bind influenza. IgM responses have also been generated against HIV proteins and other naive antigens under certain cytokine and adjuvant combinations. This platform provides many opportunities to understand and manipulate the cellular interactions that occur during an adaptive immune response, and will enable translational applications such as high throughput vaccine design and adjuvant testing.

Presenter: Wagner, Catriona

Myelin-Specific CD8+ T cells Exacerbate CNS Autoimmunity and Increase Clinical Signs of Brain Inflammation.

Catriona Wagner, Joan Goverman

University of Washington

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system (CNS). Although the pathogenic pathways of MS are not fully understood, myelin-specific T cells are hypothesized to initiate disease. Myelin-specific CD4+ T cells have been the main focus of MS research and animal models of MS have relied exclusively on the activity of CD4+ T cells. However, substantial data from MS patients indicate that CD8+ T cells also contribute to MS pathogenesis. We hypothesized that recruitment of myelin-specific CD8+ T cells to the site of inflammation initiated by CD4+ T cells will influence disease. To test our hypothesis, we use a mouse model of MS, experimental autoimmune encephalomyelitis (EAE) in which we can investigate the interplay between myelin-specific CD4+ and CD8+ T cells when disease is initiated by adoptive transfer of CD4+ T cells specific for myelin oligodendrocyte glycoprotein (MOG). To determine the influence of CD8+ T cells, we transferred MOG-specific CD4+ T cells into wild-type mice that also received an injection of naÏve CD8+ T cells from TCR-transgenic mice that express a TCR specific for a MHC class I-restricted epitope of myelin basic protein (MBP). We found that the recruitment of the MBP-specific but not control CD8+ T cells increased the incidence and severity of symptoms associated with brain inflammation. Increased disease severity was associated with a higher number of activated MOG-specific CD4+ T cells within the brain at peak disease. Furthermore, an increased number of GM-CSF- and TNFa-producing MOG-specific CD4+ T cells were detected in the brain of these mice. MBP-specific CD8+ T cells produced more TNFa in the brain and spinal cord as compared to WT CD8+ T cells, suggesting a potential mechanism by which MBP-specific CD8+ T cells exacerbate disease. These data suggest that the interplay between CD4+ and CD8+ T cells specific for two different myelin proteins is critical for determining the manifestation of CNS autoimmune disease.

Presenter: Wang, Ziming

Diet-induced obesity promotes tumor growth but greater responsiveness to PD-1 blockade

Ziming Wang, Ziming Wang, Jesus I. Luna, Ethan G. Aguilar, Lam T. Khuat, Arta M. Monjazeb, William J. Murphy

University of California Davis

T cell exhaustion has been observed in the tumor microenvironment. Exhaustion involves increased expression of inhibitory receptors, decreased cytokine production and impaired cytolytic activity, which lead to the inability to eliminate cancer. PD-1 is a critical marker of T cell exhaustion. The blockade of the PD-1/PD-L1 axis can augment T cell responses and result in significant responses across a wide spectrum of cancers, which has dramatically altered the landscape of immunotherapy in cancer. Despite the success of checkpoint blockade, a significant percentage of patients still do not respond to therapy. The goal of this study is to determine the impact of obesity on cancer outcome with a focus on T cells and the anti-tumor effects of PD-1 blockade in tumor models. We generated lean and diet-induced obese (DIO) C57BL/6 mice by feeding mice 60% vs 10% fat diets starting at age 6 weeks. 6-month DIO mice had an average body weight of 50 g compared to the 35 g average of lean mice. DIO and lean mice were implanted with 106 B16 cells subcutaneously. The B16 tumors grew significantly faster in the DIO mice compared to the lean mice. There was increased fluorodeoxyglucose (FDG) uptake in the tumors of DIO mice as determined by PET/CT indicating increased metabolic activity. Importantly, a significantly higher proportion of tumor-infiltrating CD8 and CD4 T cells in DIO mice expressed PD-1, which reflects the exhaustion of T cells in DIO tumor bearing mice. This exhaustion phenotype was also demonstrated using RNAseq analysis on sorted CD44hi T cells (purity >95%) from the spleen and lymph nodes. Histological assessment of B16 tumors excised at 21 days post-inoculation showed intact epidermis in the lean mice, while the tumors in DIO mice caused epidermis erosion and necrosis with the tumor invading the subcutaneous fat. The administration of the antagonist monoclonal antibody to PD-1 led to marked reduction of tumor burden only in the DIO mice, not the lean mice, with no toxicity observed. This decrease in tumor burden inversely correlated with tumor infiltrating CD3+ T cells. These results have implications for the efficacy of PD-1 blockade in different metabolic environments.

Presenter: Wei, Spencer

Loss of negative costimulation permits aberrant T cell differentiation

Spencer Wei, Roshan Sharma, Nana-Ama Anang, Jacob Levine, Yang Zhao, Jing Wang, Dana Pe'er, James Allison

The University of Texas MD Anderson Cancer Center

ABSTRACT TEXT NOT AVAILABLE ONLINE

Presenter: Wiesner, Darin

Epithelial club cell junction proteins regulate calcineurin signaling and allergic asthma in response to protease allergens

Darin Wiesner, Bruce S Klein

University of Wisconsin - Madison

Asthma will soon afflict a tenth of the world's population. Although fungi and their proteases account for most cases of allergic asthma, the mechanisms by which they provoke allergic inflammation remain ill defined. Here, we show that epithelial club cells lining the bronchioles sense and regulate the allergic response to Aspergillus protease. Protease disrupts the junctions between club cells in the conducting airways; in particular, cleaving the extracellular domain of e-cadherin that maintains adherens junction integrity of these cells. The loss of tensile force between adjoining club cells triggers mechanotransduction via the gated calcium ion channel, TRPV4, resulting in calcium flux, calcineurin activation, and translocation of NFATc3 or NFATc4 from the cytoplasm into the nucleus. Club cells rapidly transcribe chemokine ligand 2, recruiting monocyte-derived dendritic cells and initiating Th2 cell-dependent eosinophilia in the lungs. These data reveal a new paradigm with novel molecular targets and signaling events in lung stromal cells that recognize a common allergen and incite allergic airway disease.

Strategic Design of a live attenuated virus as a vaccine for tumor-associated herpesvirus

Ting-Ting Wu, Gurpreet Brar, Nisar Farhat, Alice Sukhina,Yong Hoon Kim, Tiffany Hsu, Sara Su, Marcia Blackman, Ren Sun

UCLA

Conventional vaccine strategies against tumor-associated herpesviruses have resulted in disappointing preclinical efficacies. The vaccination goal for these persistent pathogens is to prevent the establishment of long-term latency, which can lead to tumor development. Attenuation of a vaccine is often accompanied by a reduction in immunogenicity. In this study, we propose eliminating immune antagonist genes in order to attenuate tumor-associated herpesviruses while maintaining robust immunogenicity. This vaccine demonstrates no measurable replication capacity in immunocompetent (C57BL/6) and immunosuppressed (SCID) hosts. The attenuation is driven by the presence of the type I IFN response (IFN). At the same time, this vaccine strategy is able to elicit robust inflammatory cytokine responses, drive the differentiation of antiviral memory T cells. This study presents a vaccine strategy able to protect against tumor-associated herpesviruses from establishing long-term latency

Presenter: Wu, Xia

Precision-cut human liver slice cultures as an immunological platform

Xia Wu, Jessica B. Roberto1, Allison Knupp1, Heidi L. Kenerson2, Camtu D. Truong1, Sebastian Y. Yuen1, Katherine J. Brempelis1, Marianne Truffaux3, Antony Chen3, Helen Horton3, Raymond S. Yeung2 and Ian N. Crispe1

1Department of Pathology, University of Washington, Seattle, WA, 2Department of Surgery, University of Washington, Seattle, WA, USA, 3Infectious Diseases and Vaccines, Janssen Research and Development, B-2340 Beerse, Belgium.

The liver is the central metabolic organ in the human body, and also plays an essential role in innate and adaptive immunity. While mouse models offer significant insights into immune-inflammatory liver disease, human immunology differs in important respects. To improve the understanding of human liver immunobiology and pathology, we have established precision-cut human liver slices to study innate immunity in human tissue. Human liver slices collected from resected livers could be maintained in ex vivo culture over a two-week period. Although an acute inflammatory response accompanied by signs of tissue repair was observed in liver tissue following slicing, the expression of many immune genes stabilized after day 4 and remained stable until day 15. Remarkably, histological evidence of pre-existing liver diseases was preserved in the slices for up to 7 days. Following 7 days of culture, exposure of liver slices to the toll-like receptor (TLR) ligands, TLR3 ligand Poly-I:C and TLR4 ligand LPS, resulted in a robust activation of acute inflammation and cytokine genes. Moreover, Poly-I:C treatment induced a marked antiviral response including increases of interferons IFNB, IL-28B and a group of interferon-stimulated genes. Current effort is to use antibody blockade to manipulate cytokine production and modulate tissue repair process ex vivo in human liver slices.

Radiation Therapy as an Endogenous Vaccine

Lauren Zebertavage, Alejandro Alice, Marka Crittenden, Michael Gough

Oregon Health and Science University

Radiotherapy (RT) is one of the three main arms of traditional cancer therapy, but its role as an immunotherapeutic agent has not been fully characterized. In our murine model of pancreatic adenocarcinoma expressing model antigen SIY (Panc02-SIY), we have observed CD8-dependent clearance of tumors given single, high-dose radiation (20Gy) and anti-PD1 checkpoint blockade. Notably, we have observed inefficacy of radiation + checkpoint blockade to induce tumor clearance when T cell priming is blocked during tumor challenge (by αCD40L antibody, FTY720 or CD8 depletion). We therefore hypothesized that the role of RT was to boost existing CD8 T cell responses against tumor antigens and drive an influx of tumor-reactive cytotoxic CD8 T cells into the microenvironment, as opposed to priming new responses against tumor antigens. We found that boosting tumor-reactive T cell numbers in the tumor with a live-attenuated Listeria monocytogenes (Lm) vaccine against a major tumor antigen (SIYRYYGL, or SIY) was not sufficient to replicate the efficacy of RT when combined with checkpoint blockade. Mice treated with LmSIY and αPD1 showed no tumor growth control advantage when compared to controls, despite having up to two-thirds of CD8 T cells in circulation responding to the tumor antigen. Immunohistochemistry of embedded Panc02-SIY tumors indicate that Lm vaccination does drive T cell infiltration into the tumor, and in vitro studies show that LmSIY-induced T cells are capable of responding and secreting cytokines in response to Panc02-SIY. Additionally, in vivo specific lysis experiments show Lm-peptide vaccines are capable of inducing CD8 T cell responses capable of rapidly killing peptide-pulsed cell targets in circulation, indicating that failure of this therapy is tumor-specific. Significantly, in vitro T cell killing assays indicate that SIY-expressing Panc02 requires pretreatment (i.e. with IFNγ) in order for Listeria-SIY-primed CD8s to effectively kill. Our results indicate that the efficacy of RT in the context of checkpoint blockade cannot be replicated by boosting the number of tumor-reactive CD8 T cells within the tumor, and that the role of radiation in effective CD8-mediated therapy of immunogenic tumors may be modification of the tumor cells themselves to increase susceptibility to killing by pre-existing tumor-specific T cells.

Presenter: Zhu, Yanfang

Time-of-Flight Cytometry and Single-Cell Analysis Uncovers Unipotent Hematopoietic Neutrophil Lineage in Adult Mouse and Human

Yanfang Zhu, Paola Marcovecchio1, Amy Blatchley1, Lindsey Padgett1, Runpei Wu1, Erik Ehinger1, Cheryl Kim2, Zbigniew Mikulski1, Gregory Seumois3, Ariel Madrigal3, Huy Dinh1, Pandurangan Vijayanand3, and Catherine C. Hedrick1*

1Division of Inflammation Biology, La Jolla Institute for Allergy and Immunology, La Jolla, CA 2Flow Cytometry Core Facility, La Jolla Institute for Allergy and Immunology, La Jolla, CA 3Division of Vaccine Discovery, La Jolla Institute for Allergy and Immunology, La Jolla, CA

Neutrophils are short-lived immune cells that play important roles in a variety of diseases. The oligopotent Granulocyte Monocyte Progenitors (GMPs) in the bone marrow give rise to monocytes and all granulocytes; however, the unipotent neutrophil lineage hematopoiesis has not been well established. Here, we use Cytometry by Time-of-Flight (CyTOF) and Single-cell RNA-Sequencing (scRNA-Seq) tools to identify a CD117+ (c-Kit+) Ly6A/E- (Sca1-) Siglec F- FceRIa- CD16/32+ Ly6B+ CD11a+ (LFA1a+) CD162lo CD48lo Ly6Clo CD115- Ly6G- early-stage progenitor population in adult mouse and a heterogeneous hCD117+ hCD66b+ hCD34+/- progenitor population in human bone marrow with in vivo unipotent neutrophil potency. A Ly6Glo immediate downstream late-stage neutrophil precursor is also identified by viSNE high dimensional automated mapping. This discovery should allow for comprehensive understanding of neutrophil lineage hematopoiesis and assist discovery of important new therapeutic targets for granulopoiesis-related diseases such as in cancer.