

Presenter: Acharya, Mridu

alpha(v) Integrins Regulate B cell TLR responses through components of the autophagy pathway

Mridu Acharya, Mridu Acharya, Anna Sokolovska, Jenny Tam, Kara Conway, Raminik Xavier, Marianela Feliu, Jatin Vyas, Lynda M. Stuart, Richard O. Hynes and Adam Lacy-Hulbert

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Immune responses to apoptotic cells are tightly regulated and defects in clearance of dying cells are linked to autoimmunity. The alpha(v) integrins function as receptors for apoptotic cell recognition and phagocytosis, but how they regulate immune responses remains unknown. Here we describe a novel regulatory role for alpha(v) integrins in autoreactive B cells. We have found that mice lacking alpha(v) in B cells have increased activation of marginal zone (MZ) and B1 B cells. These mice develop auto-antibodies and are prone to lupus-like autoimmunity. In further investigation we find that alpha(v)beta3 integrin regulates toll-like receptor (TLR) signaling in MZ and B1 cells by engaging the autophagy machinery. In B cells expressing alpha(v)beta3, after stimulation, internalized TLRs rapidly become localized into vacuoles marked with autophagy components, including LC3. This correlated with reduction of the levels of active signaling components downstream of TLRs, including MAPKs and NF- κ B. In contrast, in B cells lacking alpha(v), TLRs did not colocalize with autophagy components and cells showed prolonged TLR signaling and increased proliferation. Similar increased B cell responses were seen in cells lacking key components of the autophagy machinery, strongly implicating autophagy in regulation of B cell responses. Our data point to an important mechanism of regulation of MZ and B1 B cell responses to self-antigens by alpha(v) integrin acting on TLRs such as TLR9 and TLR7. We propose a model in which alpha(v)beta3 binding to apoptotic debris induces selective autophagy of the TLR signaling components to limit autoreactive B cell responses. In ongoing work, we are identifying signaling pathways that link alpha(v)beta3 and induction of autophagy.

Presenter: Asfaw, Endale Tadesse

High prevalence of anti-hepatitis E virus among Egyptian blood donors

Endale Tadesse Asfaw, Lobna Metwally and Alaa EL- Din Saad Abd-El Hamid

Hawasa University

Not to be placed on the website.

Presenter: Barry, Kevin C.

Translation inhibition by bacterial pathogens: link to host inflammation.

Kevin C. Barry, Mary F. Fontana, Jonathan L. Portman, Aisling S. Dugan, and Russell E. Vance

University of California, Berkeley

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Presenter: Becklund, Bryan

Rapid proliferation of memory phenotype CD4⁺ T cells is primarily driven by self-antigens

Bryan Becklund, Charles D. Surh

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The memory phenotype (MP) CD4⁺ T cell population is a heterogeneous pool of cells produced upon exposure to foreign antigens and through homeostatic proliferation during transient periods of lymphopenia. Previous studies aimed at identifying factors responsible for regulating the homeostasis of MP CD4⁺ T cells have shown that a subset of MP CD4⁺ T cells undergoes a rapid rate of proliferation that is dependent on TCR interactions with peptides bound to MHC-II. However, it is unclear whether the rapidly proliferating MP CD4⁺ T cells are responding to foreign or self-derived peptide antigens. To address this question, we have analyzed the proliferation of adoptively transferred MP CD4⁺ T cells in a host which predominantly displays a single self-peptide bound to MHC-II and in germ-free hosts which have a dramatically reduced foreign antigen load. Our results indicate that self-peptide/MHC-II complexes are primarily responsible for driving the rapid proliferation of MP CD4⁺ T cells in a lymphopenic host. The vigorous response to self-antigens and acquisition of effector functions by the rapidly dividing MP CD4⁺ T cell subset could potentially play a role in mediating the breakdown in peripheral self-tolerance and induce lymphopenia-associated autoimmune disease.

Presenter: Bronevetsky, Yelena

Investigation into the mechanisms regulating human fetal T cell differentiation.

Yelena Bronevetsky, Mike McCune

UCSF

Newborns and infants are susceptible to a range of serious infections. Though this has been attributed to ineffectiveness of the developing immune system, evidence now suggests that the fetal immune system is highly effective. Rather than generating sterilizing immunity, the mandate of the fetal immune system is to generate tolerance. Ongoing experiments indicate that the fetal and adult immune systems layer upon one another such that there is an admixture of the two at the time of birth. It may be possible to change the mixture such that it is more immunoreactive and thus more likely to respond to vaccination. The flip of this switch would be akin to a natural agonist and might enhance the response of the newborn to immunizations. We hypothesize that Lin28b, a suppressor of microRNA biogenesis, regulates the switch between tolerant fetal T cell lineages and immunoreactive postnatal T lineages. Our preliminary data has determined that modulating Lin28b function in human fetal naive T cells alters their transcriptional profile and their functional properties, leading to decreased regulatory T cell differentiation.

Presenter: Burt, Trevor

Evidence for Layered Maturation of Human Fetal T Cells

Trevor Burt, Elizabeth Krow-Lucal, Christopher Baker, Norman Jones, Ekaterina Maidji, Jeff Mold, Cheryl Stoddart, Joseph (Mike) McCune

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Human fetuses and newborns are susceptible to severe infection by a wide range of pathogens, including those that are not normally harmful in adults. They also mount relatively dampened cellular and humoral responses to vaccination compared to adults. Though often attributed to inactivity, immaturity, or ineffectiveness of the developing immune system, evidence suggests that the developing human fetal immune system is highly active, but that its primary default program is to generate tolerance. Thus, the human fetal immune system is derived from multilineage hematopoietic stem/progenitor cells (HSPCs) that give rise to tolerogenic regulatory T cells while the adult immune system is derived from distinct HSPCs that are more likely to give rise to immunoreactive T effector cells. We hypothesize that different neonates may have varying proportions of these two compartments at birth, and that those with a higher proportion of the fetal compartment preferentially mount tolerogenic responses that might predispose them to serious infection or ineffective vaccine responses. To address this hypothesis, fetal and adult-specific T cell gene signatures were identified based on previous transcriptional profiling by microarray. Using these signatures, we demonstrate intermediate gene expression patterns in a cross-section of umbilical cord blood (UCB) samples at full-term. Furthermore, there was a wide range of variability in the transition from a fetal signature to one that is more adult in nature, suggesting that some infants have potential for more adult-like T cell responses. To address whether intermediate expression of fetal and adult genes at birth represents the presence of two different fetal and adult cell populations, both cytopin immunocytochemistry and single cell FACS sorting and qRT-PCR were performed. Both analyses suggest dichotomous expression of fetal and adult genes in different cell populations in full term umbilical cord blood, providing support for the existence of two distinct populations.

Presenter: Capece, Tara

Regulation of integrin LFA-1 during T cell activation

Tara Capece, Anna Abid, Minsoo Kim

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T cells spend the majority of their time perusing lymphoid organs in search of cognate antigen presented by antigen presenting cells (APCs) and quickly recirculating through the bloodstream to another lymph node. Extravasation into a given lymph node occurs through high endothelial venules (HEV) and is dependent upon several key events, including activation of chemokine receptor CCR7 by chemokine CCL19/21 and adhesion of integrin LFA-1 (CD11a/CD18) to its ligand ICAM-1. T cells must become highly migratory to scan the densely packed organ for their cognate antigen on APCs, utilizing active LFA-1 at the leading edge of a migrating T cell. Upon antigen challenge, T cell forms a stable interaction with the APC, which is known as the immunological synapse (IS). Active LFA-1 is relocated to the IS, stabilizing the interaction between the T cell and APC. Although LFA-1 is a key adhesive force for both migration and IS formation, the outcome of chemokine and T cell receptor (TCR) signaling is quite different, as the former induce go and the latter mediate stop signals. We hypothesize that the magnitude of chemokine and TCR signals received by the T cell will determine the outcome of LFA-1 mediated interactions and thus T cell activation. To investigate the role of LFA-1 during T cell activation and migration, we generated fluorescent knock-in (KI) mice that will allow us to visualize expression, distribution, and activation patterns of LFA-1. Having mated these KI mice with OTI TCR transgenic mice, we have begun studying LFA-1 redistribution and activation in T cells activated by varying TCR signal strength with altered peptide ligands (APL). Thus far, we have shown that LFA-1 clusters faster at the contact site between CD8⁺ T cells and dendritic cells (DCs) pulsed with cognate antigen compared to those pulsed with a weaker APL. Additionally, LFA-1 remains clustered at contact site longer with cognate antigen than the APL. We will repeat these experiments in our LFA-1 FRET mice, which allow us to visualize the activation status of LFA-1, to determine if LFA-1 activation induces the LFA-1 clustering seen in interactions with cognate antigen. We will also incorporate a photoactivatable chemokine receptor, PA-CCR7, to assess the effect of chemokine signal strength on LFA-1 activation.

Presenter: Carrette, Florent

Hyaluronic acid/CD44 interactions control CD8 T cell memory development to influenza virus infection.

Florent Carrette, Florent Carrette, Erin Mueller, Monique Barraza, Barbara Carrette, Linda Bradley

Sanford Burnham Medical Research Institute

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Presenter: Casbon, Amy-Jo

Distinct local and systemic regulation of myeloid cells in cancer

Amy-Jo Casbon, Damien Reynaud, Emmanuelle Passegue, and Zena Werb

University of California San Francisco

There is a growing interest in the contribution of myeloid cells that suppress T cell function in cancer progression and more recently, their role in promoting lung metastasis. However, the markers utilized to study these myeloid-derived suppressor cells (MDSCs) are shared with myeloid cells found during normal homeostasis and controversy exists as to whether these cells are a unique cell population, alternatively activated myeloid cells, or immature myeloid cells blocked during differentiation. Multiple studies have suggested that MDSCs arise from a block in differentiation, yet relatively no data exist supporting this hypothesis. Using a mouse model of breast cancer that closely mimics the stages of tumor progression observed in human patients, we took a novel comprehensive approach to determine the molecular and cellular mechanisms that regulate MDSCs in tumor-bearing mice by evaluating changes in both mature and immature myeloid cell populations in multiple tissues in longitudinal studies. Using this approach we identified the cellular and molecular mechanism regulating MDSC development was the result of tumor reprogramming of hematopoiesis in both the BM and spleen, which skewed myeloid differentiation at the level of the primitive multipotent progenitor. We also identified distinct regulatory mechanisms for local expansion of tumor-associated macrophage-dendritic (M-DC) populations and systemic expansion of MDSCs. In addition, our longitudinal studies revealed that changes in subpopulations of tumor M-DCs occurred at the very onset of tumor development during hyperplasia, but changes in systemic myeloid cells were not observed until late stage tumor progression and appeared to parallel malignant conversion. In summary, our study has enhanced our understanding of the mechanisms by which tumors regulate the immune system in cancer and the systemic effects of cancer that promote tumor growth in distant metastatic sites. Supported by funds from the NCI (R01 CA057621) and NIH (T32 CA108462).

Presenter: Chavarria-Smith, Joseph

Direct proteolytic cleavage of NLRP1B is necessary and sufficient for inflammasome activation by anthrax lethal factor

Joseph Chavarria-Smith, Russell E. Vance

University of California, Berkeley

Inflammasomes are multimeric protein complexes that respond to infection by recruitment and activation of the Caspase-1 (CASP1) protease. Activated CASP1 initiates immune defense by processing inflammatory cytokines and by causing a rapid and lytic cell death called pyroptosis. Inflammasome formation is orchestrated by members of the nucleotide-binding domain and leucine-rich repeat (NLR) or AIM2-like receptor (ALR) protein families. Certain NLRs and ALRs have been shown to function as direct receptors for specific microbial ligands, such as flagellin or DNA, but the molecular mechanism responsible for activation of most NLRs is still poorly understood. Here we determine the mechanism of activation of the NLRP1B inflammasome in mice. NLRP1B, and its ortholog in rats, is activated by the lethal factor (LF) protease that is a key virulence factor secreted by *Bacillus anthracis*, the causative agent of anthrax. LF was recently shown to cleave mouse and rat NLRP1 directly. However, it is unclear if cleavage is sufficient for NLRP1 activation. Indeed, other LF-induced cellular events have been suggested to play a role in NLRP1B activation. Surprisingly, we show that direct cleavage of NLRP1B is sufficient to induce inflammasome activation in the absence of LF. Our results therefore rule out the need for other LF-dependent cellular effects in activation of NLRP1B. We therefore propose that NLRP1 functions primarily as a sensor of protease activity and thus could conceivably detect a broader spectrum of pathogens than just *B. anthracis*. By adding proteolytic cleavage to the previously established ligand-receptor mechanism of NLR activation, our results illustrate the remarkable flexibility with which the NLR architecture can be deployed for the purpose of pathogen-detection and host defense.

Presenter: Clingan, Jonathan

Regulation of antiviral immune responses by RNase L

Jonathan Clingan, Sterling Eckard, Daniel Stetson

University of Washington

Recognition of nucleic acids by innate immune sensors is a common mechanism by which an immune response is initiated. In particular, during virus infection, cytosolic RNA can trigger a potent innate immune response via RIG-I-like receptors (RLRs) characterized by a massive increase in the expression of antiviral type I interferons (IFNs) and interferon-stimulated genes (ISGs). Intracellular dsRNA ligands can also activate oligoadenylate synthetase (OAS) to produce 2', 5'-oligoadenylates which activate RNase L. Cleavage of viral and host RNA substrates by RNase L has generally been thought to limit virus replication and contribute to type I IFN responses. However, our studies have revealed a role for RNase L as a negative regulator of the host antiviral immune response. Additionally, RNase L targets ribosomal RNA (rRNA) in a specific manner, but how cleavage of rRNA by RNase L contributes to translational regulation is unknown.

Presenter: Collin, Roxanne

Mouse *Idd2* locus is linked to the proportion of immunoregulatory DN T cells, a trait associated with autoimmune diabetes resistance

Roxanne Collin, Veronique Dugas, Adam-Nicolas Pelletier, Genevieve Chabot-Roy, Sylvie Lesage

Universite de Montreal

Autoimmune diseases, such as type 1 diabetes (T1D), result from a break in immune tolerance leading to an attack on self-antigens. Various mechanisms of peripheral tolerance can protect against autoimmunity, including immunoregulatory CD4-CD8⁻ (double negative, DN) T cells. Indeed, using the 3A9 TCR:iHEL transgenic mouse model, we have shown that diabetes-prone strains exhibit a low proportion of DN T cells relative to that of diabetes-resistant mice and that a single autologous transfer of DN T cells can impede T1D development. Interestingly, a linkage analysis in F2 (NOD.H2k x B10.Br) mice revealed that a locus on chromosome 9 (Chr9L) is linked to DN T cell number. Thus, we hypothesized that genetic factors included within Chr9L can regulate the number of DN T cells, which may prevent T1D progression. To address this postulate, we generated congenic mice on NOD (diabetes-prone) background, where the Chr9L interval is of B10.Br (diabetes-resistant) origin. Our results show an increase in DN T cell proportion in the NOD.Chr9L congenic strain relative to the NOD parental strain. Interestingly, the increased DN T cell proportion in the NOD.Chr9L congenic strain associated with a decrease in both diabetes incidence and antigen-specific auto-antibody levels. Together, these findings suggest that a physiological increase in DN T cell number may be sufficient to confer resistance to autoimmune diabetes, potentially leading to new therapeutic avenues for T1D as well as other autoimmune diseases.

Presenter: Crowl, John

Evolutionary Tradeoffs between RNA Interference and Rig-I Like Receptors

John Crowl, Dan Stetson, Hannah Volkman

University of Washington

RNA Interference is a post-transcriptional regulatory mechanism through which genes can be silenced by the down-regulation of template matched RNAs. Most invertebrates possess an amplified RNAi response mediated by RNA Dependent RNA Polymerases (RdRPs), which generate a pool of secondary small RNAs. This allows organisms that possess RdRPs, such as the nematode *C. elegans*, to undergo robust, heritable silencing and to use RNAi as a potent antiviral defense mechanism. Despite the apparent benefits of RdRPs, these enzymes were abruptly lost early in the chordate lineage. However, most of these organisms that lack RdRPs possess an alternative means of antiviral defense in the Rig-I like receptors (RLRs), which detect the RNA products of viral RdRPs and activate a type I interferon-mediated antiviral response. We propose that sensitive detection of viral RdRP products by RLRs necessitated the abrupt loss of endogenous RdRPs during chordate evolution, along with an accompanying loss of potency in RNAi. To test this, we have introduced *C. elegans* RdRPs into mammalian cells, and we have found that one of them activates a potent type I Interferon response dependent on RLR signaling and the RdRP's catalytic activity.

Presenter: Cunningham, Cameron R.

Thymic Depletion During Persistent Viral Infection

Cameron R. Cunningham, Ivan Osokine, David G. Brooks

UCLA

The thymus is responsible for the education and maturation of functional T lymphocytes and can be targeted by some persistent infections, such as HIV, causing a dramatic loss in thymic cellularity. Interestingly, we observed severe thymocyte depletion in persistent lymphocytic choriomeningitis virus (LCMV) infection in mice. A transient and minimal loss of thymic cellularity occurred in acute LCMV infection, but the thymus was quickly replenished once virus was purged. On the other hand, prolonged virus replication during persistent LCMV infection induced an extreme loss of thymic cellularity, particularly the double positive population (CD4+CD8+), that lasted throughout the course of infection. Thymocyte depletion is dependent on LCMV-specific CD8 T cells and type I interferon signaling, and when either is absent upon infection, loss of thymic cellularity is prevented. Interestingly, thymic depletion occurred in the absence of robust direct infection of thymocytes, suggesting an indirect mechanism of thymocyte depletion and one that may be conserved even in persistent infections that do not infect thymocytes. Virus specific CD8+ T cells initially migrated to the thymus temporally correlating with thymocyte depletion. Similar to other tissues, CD8 T cells rapidly become exhausted in the thymus during persistent infection corresponding to the rebound in the normal distribution of thymocyte populations (although total thymic cellularity remains severely depressed). Yet, despite the reconstitution of thymopoiesis, no new LCMV-specific CD8 T cells were generated. We propose that persistent infection in the absence of direct thymocyte infection can lead to severe thymic depletion, the inability to produce new virus-specific T cells and thus may limit the efficacy of therapies designed to reconstitute host immunity during persistent infection.

Presenter: De Leon, Justin

Reporter cell lines as a tool to study inflammasomes

Justin De Leon, Joseph Chavarria-Smith, Russell E. Vance

University of California, Berkeley

Inflammasomes are multiprotein complexes that respond to a variety of infectious and noxious stimuli in the cytosol. Each inflammasome consists of an NLR (nucleotide binding domain, leucine rich repeat containing) protein that participates in agonist binding or interactions, a CARD (Caspase recruit domain) containing adapter, and the Caspase-1 (CASP1) protease. Activation of the inflammasome dimerizes and activates CASP1 leading to a rapid lytic cell death, called pyroptosis, and the release of pro-inflammatory cytokines. We have developed a variant of CASP1 in which the catalytic protease domain has been replaced with each of two halves of 'split' Venus, a fluorescent protein derived from GFP. Therefore, instead of pyroptosis, inflammasome activation leads to Venus dimerization and fluorescence. We have developed a number of novel reporter cell lines in 293T cells each expressing a NLR, a corresponding adapter protein, and the CASP1-Venus chimeras. We are currently utilizing these reporter cell lines in genetic screens to identify unknown inflammasome agonists and to further elucidate our structural understanding of known inflammasome agonists.

Presenter: Deshpande, Neha

The CD4 T-cell repertoire becomes poly-specific with age.

Neha Deshpande, Michael S. Kuhns

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Presenter: Dong, Han

CD27 costimulation enhances IL-7 receptor re-expression on CD8+ T cells during viral infection and promotes CD8+ T cell memory

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The development of long term memory is the major goal of vaccination, and therefore defining the factors that regulate the establishment and maintenance of memory cytotoxic CD8+ T cells is of particular interest. In many cases of viral infection, the development of fully functional CD8+ T cell memory is dependent upon helper CD4+ T cell support. CD4+ T cells play a critical role in inducing the expression of CD70, the ligand for CD27, on dendritic cells (DCs). In a vaccinia infection model, our previous data demonstrated that 1) the defects in CD8+ T cell memory that occur in the absence of CD4+ helper T cells were a consequence of inadequate CD27 costimulation; and 2) CD27 costimulation led to increased interleukin 7 receptor A (IL-7RA) on CD8+ T cells. IL-7 plays a critical role in the generation and long-term maintenance of memory CD8+ T cells; and in fact increased IL-7RA has been regarded as a defining marker to long-lived memory precursor effector cells. CD27 costimulation promotes CD8+ T cell memory presumably via enhancing the abundance of IL-7RA-expressing memory precursor pool. Little is known about the factors that promote the expression of IL-7R in Ag-activated CD8+ T cells. Here we explore the underlying molecular mechanism by which CD27 costimulation enhances IL-7RA-expressing memory precursors and show that CD27 signal facilitates the re-expression of IL-7RA on CD8+ effector T cells. Naive CD8+ T cells uniformly express IL-7RA. Stimulating CD8+ T cells by antigen (Ag) peptide in vitro resulted in down-regulation of the IL-7RA within 48hr, which could not be alleviated by CD27 costimulation. This indicates that CD27 costimulation during early priming phase does not prevent IL-7RA down-regulation upon TCR engagement. After adoptively transferring IL-7RA-down-regulated CD8+ T cells from culture to cognate Ag-infected mice, however, stimulation of CD27 via agonistic antibody led to a dramatic increase in frequency and absolute number of IL-7RA-expressing CD8+ T cells, implying late CD27 costimulation supports IL-7RA re-expression. Importantly, CD27 costimulation promotes not only IL-7RA, but also the common γ chain of the receptor and the downstream signaling mediated by pSTAT5. Our current studies indicate that CD27 costimulation facilitates IL-7RA re-expression by direct regulation rather than via promoting the expansion/survival of IL-7R-expressing cells. Data will be presented determining how IL-7RA is regulated, particularly whether CD27 costimulation induces mRNA transcription or modulates surface protein turnover and how. Our results provide insights into the mechanistic basis by which CD27 costimulation influences CD8+ T cell memory differentiation, and highlight the potential of targeting CD27-CD70 axis to enhance IL-7 signaling for antiviral/antitumor immunotherapy.

Presenter: Duval, Frederick

Context-dependent role for the Notch signaling pathway in CD8+ T cell response

Frederick Duval, Melissa Mathieu, Nathalie Labrecque

University of Montreal

Following an infection, naive CD8 T cells expand and differentiate into effectors able to eliminate the pathogen. At the peak of the response, two major populations of effectors are distinguishable: short-lived effector cells (SLECs) meant to die by apoptosis and memory precursor effector cells (MPECs) destined to survive as memory cells that will confer long-term protection. Thus, we have postulated that the Notch signaling pathway, known for its role in cellular differentiation and binary cell fate choice, acts as a key player in the MPEC/SLEC differentiation choice. To elucidate the role of Notch signaling in CD8 T cell response, we analyzed CD8 T cell response in mice lacking or not the expression of Notch1 and Notch2 in mature CD8 T cells. Following infection with *Listeria monocytogenes* (Lm) or vaccination with antigen-pulsed dendritic cells (DCs), Notch deficiency drastically diminished SLEC (CD127^{lo}KLRG1^{hi}) generation but did not impair the generation of functional CD8 memory T cells. However, we observed a differential effect of Notch deficiency on clonal expansion and interferon-gamma production depending on the method used to prime naive CD8+ T cells. The production of interferon-gamma by antigen-specific CD8+ T cells was severely reduced by Notch deficiency only following DC vaccination while clonal expansion was augmented in the absence of Notch only following Lm infection. We are currently investigating whether the difference in inflammation levels that occurred following Lm infection and DC vaccination explained the difference in the role of the Notch signaling pathway during CD8+ T cell response. Our results uncover an essential role for the Notch signaling pathway for SLEC differentiation and a context dependent role for this pathway in clonal expansion and interferon-gamma production by antigen-specific CD8+ T cells. A better understanding of the signals controlling CD8+ effector and memory T cell generation is essential to design better strategies to fight infection and to produce better vaccines. Funded by the Canadian Institutes of Health Research

Presenter: Dvorakova, Barbora

Determination of MTD and anti-tumor activity of novel HPMA copolymer-based drug conjugates

Barbora Dvorakova, Barbora Dvorakova[∞], Tomas Etrych, Milada Sirova[∞], Pavel Rossmann[∞], Jakub Tomala*, Blanka Rihova*, Karel Ulbrich[∞] and Marek Kovar**

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Polymeric drug conjugates based on N-(2-hydroxypropyl)methacrylamide (HPMA) belong among most intensively studied delivery systems of therapeutics as they have significantly lowered toxicity and prolonged circulation half-life in comparison to free drugs. Biocompatibility and immunocompatibility of HPMA copolymer makes it almost ideal drug carrier together with its ability to carry multiple pendant groups due to several functional groups present on the polymer backbone. Novel HPMA-based polymeric conjugates bearing anthracycline antibiotic doxorubicin (HPMA-DOX) bound via hydrazone bond were tested for their biological activity in vivo in terms of maximum tolerated dose (MTD) and anti-tumor activity. Two types of conjugates differing in structure were compared, i.e. linear (Mw ~ 27 000) and star-like (Mw ~ 250 000) conjugates. Their MTD was tested both on Balb/c and on C57BL/6 mice and determined to be 85 mg/kg DOX and 22.5 mg/kg DOX, respectively. Anti-tumor activity of these conjugates was measured on murine B-cell leukemia (BCL1) and mouse mammary carcinoma (4T1) for dosage of 100% and 60% of MTD, and on mouse T-cell lymphoma (EL4) for dosage of 100%, 60% and 30% of MTD. In case of BCL1 and EL4, significantly prolonged survival or even complete cure of several experimental mice was observed. The positive effect of combined therapy of BCL1 leukemia by star-like HPMA-DOX conjugate and regulatory T cell depletion was also investigated. This work was supported by Czech Science Foundation grant P301/11/0325, 13-12885S and by Institutional Research Concept RVO 61388971.

Presenter: Eckard, Sterling

The SKIV2L RNA Exosome Limits Activation of the RIG-I-Like Receptors

Sterling Eckard, Daniel B. Stetson

University of Washington

All cells have sensors that detect foreign nucleic acids and trigger a cell-intrinsic antiviral response through the production of type I interferons. In addition to viral ligands, endogenous nucleic acids can potentially activate these same sensors and elicit an identical antiviral response, leading to autoimmune disease. In the case of the DNA-activated antiviral response called the interferon-stimulatory DNA (ISD) pathway, the Trex1 cellular DNA exonuclease metabolizes the DNA ligands that trigger the ISD sensors. Trex1-deficient humans develop a severe, lupus-related autoimmune disease called Aicardi-Goutieres Syndrome (AGS), demonstrating the importance of removing these DNA ligands from within cells. In contrast to the Trex1/ISD pathway regulation, it is unknown whether a similar mechanism exists to limit the RIG-I and MDA5-mediated antiviral response to RNA. We found that the 3'→5' RNA exosome, a multiprotein complex involved in cellular RNA turnover, is a potent negative regulator of the RIG-I/MDA5 pathway. Knockdown of SKIV2L, a component of the RNA exosome and a recently described lupus susceptibility allele, resulted in massive enhancement of the antiviral response to RNA ligands. Moreover, rare human mutations in RNA exosome components cause a disease called Tricho-Hepato-Enteric (THE) syndrome, and patients with THE syndrome have evidence for aberrant immune activation. Together, these findings identify a key RNA degradation pathway that regulates the RIG-I/MDA5-mediated antiviral response and demonstrate that metabolism of endogenous, intracellular nucleic acids is a key mechanism for preventing inappropriate activation of both DNA and RNA sensors.

Presenter: Feigelson, Sara

Resting and inflamed blood vessels pattern distinct heparan sulfate gradients between their apical and basolateral aspects

Sara Feigelson, Liat Stoler-Barak, Katya Kopitman, Ronen Alon
Weizmann Institute of Science

A hallmark of immune cell trafficking across endothelial cells of post capillary venules is directional guidance via gradients of soluble or glycosaminoglycan (GAG)-presented chemokines. Endothelial heparan sulfate (HS) proteoglycans were suggested to be a critical GAG scaffold for chemokines and also important for the barrier properties of the endothelial basement membrane. It is unclear how endothelial GAGs sustain steep chemokine gradients between the luminal and basolateral aspects of blood vessels. To address these and related questions, we analyzed HS distribution around post capillary venules of either resting or inflamed skin as well as of skin draining lymph nodes. We find that the blood vasculature patterns steep gradients of HS scaffolds between their luminal and basolateral endothelial aspects in skin venules as well as in high endothelial venules (HEVs) of lymph nodes. Skin inflammation but not lymph node inflammation results in massive ECM deposition and in further HS enrichment around post capillary venules. Notably, although effector T cells express high levels of the main HS degrading enzyme, heparanase, they do not need it to attach, extravasate and cross the HS rich basement membranes of either lymph node HEVs or inflamed skin post capillary venules. Our results suggest that lymphocytes do not need heparanase to disrupt HS moieties within basement membranes, possibly because they may recognize ECM discontinuities permissive for basement membrane crossing. Thus, rather than generating a barrier for leukocyte extravasation, enriched HS proteoglycans within the basement membranes of blood vessels are necessary to maintain steep chemokine gradients between the luminal and basolateral aspects of these vessels.

Presenter: Fu, Guo

Themis sets the signal threshold between positive and negative selection in T-cell development

Guo Fu, Javier Casas, Stephanie Rigaud, Vasily Rybakin, Florence Lambolez, Joanna Brzostek, John Hoerter, Wolfgang Paster, Oreste Acuto, Hilde Cheroutre, Karsten Sauer, Nicholas Gascoigne

The Scripps Research Institute

Development of a self-tolerant T-cell receptor (TCR) repertoire with the potential to recognize foreign antigens depends on proper regulation of TCR signaling, which is initiated by its interaction with self-peptides presented by major histocompatibility complex proteins (MHCp). Current model in the field stated that low-affinity TCR interactions with self-MHCp generate weak but sustained signals that trigger positive selection, causing maturation of CD4 or CD8 $\alpha\beta$ -expressing single positive (SP) thymocytes from CD4+CD8 $\alpha\beta$ + “double positive” (DP) precursors. These SP thymocytes further develop into mature naive T-cells of the secondary lymphoid organs. In contrast, TCR interaction with high-affinity agonist self-ligands results in “negative selection” by activation-induced apoptosis, or “agonist selection” of functionally differentiated self-antigen-experienced T-cells. Here we show that positive selection is enabled by the ability of the T-cell specific protein Themis to specifically attenuate TCR signal strength via SHP1 recruitment and activation in response to low but not high-affinity TCR engagement. Themis acts as an analog-to-digital converter translating graded TCR affinity into clear-cut selection outcome by dampening mild TCR signals Themis increases the affinity threshold for activation, enabling positive selection of T-cells with a naive phenotype in response to low-affinity self-antigens.

Presenter: Geiger, Kristina

A Murine Model for Equine *Corynebacterium pseudotuberculosis* Vaccine Development

Kristina Geiger, Megan Sumida

Occidental College

Pigeon Fever is an equine disease caused by the bacterium *Corynebacterium pseudotuberculosis*. Our main goals are to understand the equine immune response against this bacterium and to use a mouse model to create a successful vaccine. C57Bl/6 mice are used to study the effects of a strong Th1 immune response; these are compared to BALB/c mice that exhibit a strong Th2 immune response. Mice were immunized with formalin-inactivated components of *C. pseudotuberculosis* or with phospholipase D (PLD), the major secreted exotoxin. Mice were challenged intradermally with live bacteria at a previously determined lethal dosage. ELISA analysis shows that both BALB/c and C57Bl/6 mice make a high IgG anti-PLD when vaccinated with PLD. Both strains make a high IgG response against cell lysate when vaccinated with cell lysate or cell debris. In general, the BALB/c strain produces a higher antibody response than the C57Bl/6 strain. BALB/c mice with higher IgG responses to PLD had a better survival rate than mice with lower antibody responses. Cell debris gave the best protective immune response. Overall, BALB/c mice displayed higher survival rates than C57Bl/6 mice, especially with the PLD vaccine. Monoclonal antibodies to *C. pseudotuberculosis* PLD and cell lysate proteins have been generated and are being used to identify proteins recognized by infected horses and challenged mice. Such proteins are good potential vaccine components. The identified proteins will be cloned and the recombinant proteins tested for effectiveness as protective vaccine components.

Presenter: Gerard, Audrey

Meandering motility of T cells is crucial for finding rare cognate antigen and is driven by Myosin1g

Audrey Gerard, Genaro Patino-Lopez, Peter Beemiller, Rajalakshmi Nambiar, Khadija Ben-Aissa, Yin Liu, Matthew J. Tyska, Stephen Shaw and Matthew F. Krummel

UCSF

To mount an immune response, T lymphocytes must successfully find their cognate antigen at the surface of antigen-presenting cells. But how T cells are steered to optimize their chances of encountering these antigens is unknown. T cell motility in tissues resembles a random or Levy walk and is regulated in part by external factors including chemokines and lymph node topology. We provide evidences that T cell motility and search behavior are also hard-wired. The unconventional Myosin 1g (Myo1g) motor enforces an intrinsic T cell meandering search and prolongs T-DC interactions during lymph node surveillance. Meandering motility regulated by Myo1g is cell autonomous, and is regulated in part through motor-dependent recruitment to sites of membrane perturbation and retraction. Finally, meandering motility regulated by Myo1g is crucial for T cell reactivity towards rare cognate antigen presenting cells.

Presenter: Goldberg, Emily L.

**Immune memory-boosting dose of rapamycin curtails expansion of effector immunity
against infection**

*Emily L. Goldberg, Megan J. Smithey, Lydia K. Lutes, Jennifer L. Uhrlaub, Janko Nikolich-
Zugich*

University of Arizona

Not to be placed on website.

Presenter: Gowen, Benjamin

**RBM4 and ATF4 Independently Regulate Expression of the Human NKG2D Ligand
ULBP1**

Benjamin Gowen, Caleb Marceau, Jeanmarie Gonzalez, Jan Carette, David Raulet
UC Berkeley

Not to be placed on website.

Presenter: Gray, Elizabeth

Functional Analysis of the Interferon Stimulatory DNA Pathway.

Elizabeth Gray, Dan Stetson

University of Washington

Not to be placed on website.

Presenter: Gschweng, Eric

**In vivo sr39TK PET Reporter / Suicide Gene Functionality in Human Hematopoietic Stem
Not to be placed on website. Cells**

*Eric Gschweng, Melissa N. McCracken, Michael L. Kaufman, Roger P. Hollis, Xiaoyan Wang,
Navdeep Saini, Richard Koya, Thinle Chodon, Antoni Ribas, Owen N. Witte, Donald B. Kohn*

University of California Los Angeles

Engineering immunity by lentiviral introduction of antigen-specific T-cell-receptors (TCR) into hematopoietic stem cells (HSC) for adoptive immunity can potentially generate a long-term supply of effector T-cells to eradicate malignant disease. The ability to monitor gene-modified cells in vivo and ablate them in the case of an adverse event provides crucial safety to this therapeutic modality. The dual purpose positron emission tomography (PET) reporter imaging/suicide gene modified from herpes-simplex-virus-thymidine-kinase (sr39TK) allows non-invasive assessment of engrafted gene-modified cells, as well as the ability to eliminate such modified cells in the event of on-target/off-organ reactivity or insertional oncogenesis. Here we demonstrate the in vivo imaging of human hematopoietic cells derived from G-CSF mobilized CD34 enriched peripheral-blood stem cells (PBSC) modified with a lentivirus containing the NY-ESO-1-TCR co-expressed with sr39TK in a humanized mouse model. Human cells were detected with PET reporter imaging in hematopoietic niches such as femurs, humeri, vertebrae, and thymus. Ablation of PET signal, NY-ESO-1-TCR bearing cells, and lentiviral vector elements were observed in niches upon treatment with ganciclovir (GCV), but not with vehicle control. We demonstrate that sr39TK is an efficient PET reporter and effective suicide gene for immunotherapy of TCR transduced PBSCs and their progeny in vivo.

Presenter: Han, Arnold

Linking the T cell receptor repertoire to multi-parametric phenotyping at the single-cell level

Arnold Han, Jacob Glanville, Leo Hansmann, and Mark M. Davis
Stanford University

T lymphocytes recognize a vast array of different antigens through their T cell receptor (TCR) heterodimers. They have very diverse functional activities, from stimulating B cells to make high affinity antibodies to inhibiting responsiveness. In many cases, the major specificities and functional characteristics of a T cell response are not known. Thus, we have devised a methodology by which TCR heterodimers of individual T cells can be amplified and sequenced together with genes characteristic of the different T cell types, linking function and specificity. This approach also enables the receptor to be reconstituted and used for functional studies, ligand discovery, or therapeutics. We demonstrate the utility of this approach by analyzing tumor infiltrating lymphocytes (TILs) in a colorectal carcinoma. We provide evidence that Th17-phenotype T cells are clonally expanded within tumors, are selected in an antigen-specific manner, and originate from FOXP3+ T cells.

Presenter: Hansmann, Leo

Peripheral blood CD24⁺CD38⁺CD27⁺ B cells - biomarkers for multiple myeloma

*Leo Hansmann, Lisa Blum-Scalfone, Chia-Hsin Ju, Michaela Liedtke, William Robinson, Mark
M. Davis*

Stanford University

Not to be placed on website.

Presenter: Hess Michelini, Rodrigo

Foxo1 is essential for effector-to-memory transition in CD8+ T cells

*Rodrigo Hess Michelini, Andrew L. Doedens, Carol D. Katayama, Ananda W. Goldrath and
Stephen M. Hedrick*

UCSD

The forkhead O transcription factors (FOXO) integrate a range of extracellular signals, including growth factor signaling, inflammation, oxidative stress, and nutrient availability, to substantially alter the program of gene expression and modulate cell survival, cell cycle progression, and many yet to be unraveled cell type-specific responses. Naive antigen-specific CD8+ T cells undergo a rapid expansion and arming of effector function within days of pathogen exposure. In addition, by the peak of expansion, they form precursors to memory T cells capable of self-renewal and indefinite survival. Using lymphocytic choriomeningitis virus Armstrong to probe the response to infection, we found that Foxo1^{-/-} CD8+ T cells expand normally with no defects in effector differentiation, but continue to exhibit characteristics of effector T cells long after antigen clearance. The KLRG1^{lo} CD8+ T cells that are normally enriched for memory-precursor cells retain Granzyme B and CD69 expression, and fail to up-regulate TCF7, EOMES, and other key memory signature genes, thus showing a bias toward terminal differentiation. In addition, FOXO1 directly binds an important fraction of these genes. As a correlate, Foxo1^{-/-} CD8+ T cells were virtually unable to expand upon secondary infection. Collectively, these results demonstrate an intrinsic role for FOXO1 in establishing the post-effector memory program that is essential to forming long-lived memory cells capable of immune reactivation.

Presenter: Hickle, Colin

A Toll-like receptor 9 ligand nanoparticle formulation for prophylactic vaccination against *Bacillus anthracis* (Anthrax) enhances in vivo uptake of TLR9-ligand and induction of maturation markers on antigen-presenting cell populations in mice

Colin Hickle, M. Kachura, S.A. Kell, C. Calacsan, R. Kiwan, R. Milley, G. Ott, H. Kanzler, R. L. Coffman, J.D. Campbell

Dynavax Technologies, Berkeley, CA

CpG containing oligodeoxynucleotide (CpG-ODN) sequences signal through TLR9 and are strong activators of innate immunity and highly effective adjuvants. With the aim to increase CpG-ODN adjuvant activity for more rapid and high titer antibody responses, we developed a polyvalent nanoparticle formulation of the CpG-ODN molecule DV230 through covalent linkage to the highly cross-linked sucrose polymer, Ficoll. DV230-Ficoll demonstrated a strong potency advantage over un-formulated DV230 as an adjuvant for vaccination with anthrax recombinant protective antigen (rPA) in both mice and monkeys. A single immunization with DV230-Ficoll + rPA induced rapid high titer neutralizing antibody responses and protected monkeys against aerosol challenge with 200 LD₅₀ of *B. anthracis*. As differential uptake by and induced maturation of antigen presenting cell populations may be mechanism(s) by which the nanoparticle adjuvant formulation induces more potent immunogenicity, we explored these parameters in BALB/c mice. To measure cellular uptake of the adjuvants, fluorescently labeled (A555) DV230-Ficoll or DV230 was injected into the footpads and draining lymph nodes harvested 24 and 48 hours later. Both DV230-Ficoll-A555 and DV230-A555 were detected by flow cytometry in several cell populations but DV230-Ficoll-A555 was preferentially localized to a greater extent in CD11c⁺ dendritic cell subsets. Following footpad injection with non-A555-labeled adjuvants, DV230-Ficoll was more potent in inducing expression of the maturation markers CD86 and CD69 on several cell populations, most notably on plasmacytoid dendritic cells and B cells. In summary, these data suggest that preferential uptake by and enhanced induction of maturation marker expression on antigen-presenting cell populations may be key mechanisms contributing to the potency advantage of DV230-Ficoll over un-formulated DV230 in induction of high titer neutralizing antibody responses to anthrax rPA. This project was supported with funds from NIAID/NIH/DHHS under Contract No. HHSN272200800038C. The rPA protein was provided by PharmAthene, Inc.

Presenter: Higdon, Lauren

Germinal centers are required for T cell receptor revision

Lauren Higdon, Pamela Fink

University of Washington

Tolerance of VB5+ peripheral CD4 T cells to the endogenous superantigen Mtv-8 is induced in B6 mice by one of two pathways, deletion or T cell receptor (TCR) revision. When a T cell undergoes revision, surface TCRB expression is down-regulated in response to interaction with Mtv-8, leading to induction of RAG and TdT expression, rearrangement of a new endogenous TCRB chain, and expression of the new TCRB on the cell surface. Revising T cells localize in or near germinal centers (GCs), and there is an Mtv-8 dependent increase in production of high affinity antibodies and formation of GC follicular helper T (Tfh) cells and memory B cells in VB5 Tg mice. These data led us to hypothesize that revising T cells are Tfh and that GC interactions are required for revision. Revising T cells express several Tfh-associated surface markers, including PD-1 and CXCR5, as well as the Tfh-associated transcription factor, Bcl-6. In addition, Bcl-6 is required for efficient revision, and its mutually antagonistic repressor, Blimp-1, inhibits the process. Long-lived B cell - T cell interactions mediated by the intracellular adaptor protein SAP are required for completion, but not initiation, of revision. Overall, these data demonstrate that revising T cells adopt a Tfh-like phenotype, and that GCs are required for efficient TCR revision. B cells are known to mutate their antigen receptors within GCs; our data suggest that GCs also provide a safe microenvironment for T cells to revise their TCRs.

Presenter: Hino, Christopher

**Optimizing the Purification of Mutant and Wildtype Recombinant Phospholipase D:
Approaches to producing a *C. pseudo tuberculosis* Vaccine.**

*Christopher Hino, Utsav Patwardhan, Nathan Young, Megan Sumida, Kristina Geiger, Dr.
Karen Molinder, Dr. Roberta Pollock*

Occidental College

Pigeon Fever is a major infectious equine disease caused by the gram- positive bacterium *Corynebacterium pseudotuberculosis*. The disease is dominantly pervasive in warm, arid regions within the Western United States and is often contracted through exposure to the bacteria via open wounds or fly bites. The disease manifests as external abscesses, internal infections or ulcerative lymphangitis. The less common internal infections have a mortality rate of 40%. By studying the disease we hope to characterize the equine immune response to ultimately develop a vaccine, and improve diagnostic and management tools for affected horses. The use of immunogenic proteins produced by *C. pseudotuberculosis* remains a primary component of which we hope to use in an effective vaccine. Of particular interest, Phospholipase D (PLD), the major exotoxin protein secreted by *Corynebacterium pseudotuberculosis*, plays a role in the pathogenesis of the disease. Recombinant PLD was purified via cobalt affinity chromatography and subsequently formalin inactivated. Due to decreased yields following formalin inactivation, an enzymatically inactive mutant form was purified. The purification of both recombinant wildtype and mutant PLD was optimized for a 20-fold increase in yield and a significant improvement in purity. A DNA vaccine was additionally purified using a mammalian expression vector carrying the mutant PLD. Mice were immunized with the DNA vaccine, the formalin-inactivated protein, or mutant protein. The antibody response was then measured by ELISA.

Presenter: Hoyer, Katrina

Dysregulated CD8+ T cells promote antibody-mediated autoimmune disease

Katrina Hoyer, David Gravano, P. Dominick Sanders, Sharif Sadiqi, Sara H. Isakson

University of California Merced

IFN γ -producing CD4+ and CD8+ T cells accumulate during systemic autoimmune disease in IL-2-deficient mice. CD4+ T cells are necessary for disease progression, and we have previously shown that IL-2-KO CD4+ T cells can transfer autoimmunity to recipient mice. However, the importance of CD8+ T cells to initiation and propagation of disease is unclear. Several groups have demonstrated a protective role for CD8+ T cells in inflammation and autoimmunity, while others have suggested a pathogenic role. To define the function of CD8+ T cells during spontaneous autoimmune disease, we depleted CD8+ T cells in IL-2-KO mice and evaluated the pathogenesis of autoimmunity. Elimination of CD8+ T cells for only two weeks resulted in a significant augmentation of survival, reduction in the number and activation state of the CD4+ T cells and B cells, and a delay in the development of erythrocyte (RBC)-specific antibodies. In untreated IL-2-KO mice, both CD4+ and CD8+ T cells produced IFN γ in response to RBC antigens. This upregulation indicates that autoreactive CD8+ T cells specific for RBC-antigens are present during disease. Our results suggest that autoreactive CD8+ T cells contribute to the initiation or propagation of lymphoproliferation and pathogenesis of autoimmunity. We are currently addressing the relative importance of autoreactive CD8+ T cells to that of the CD4+ T cells. We are evaluating the mechanisms by which CD8+ T cells influence the autoimmune disease process and early data indicate that IL-2-KO CD8+ T cells produce elevated levels of perforin, granzyme B and IFN γ , and induce proliferation of B cells. Supported by NIH grant 4R00HL090706-03

Presenter: Hsu, Lih-Yun

Alteration of T cell signaling strength by ZAP-70 mutants reveals a signaling threshold for Th17 versus Treg cell differentiation

Lih-Yun Hsu, Debra Cheng, Yiling Chen, Arthur Weiss

UCSF

Not to be placed on website.

Presenter: Huang, Yanping

Regulation of T-cell adhesion and migration by Crk family adaptors

Yanping Huang, Taku Kambayashi, Janis K. Burkhardt

The Children's Hospital of Philadelphia

T cells are critical mediators in many chronic inflammatory responses, including graft vs. host disease (GVHD). These disease conditions depend on the effector functions of T cells, and their abilities to migrate to the sites of inflammation. Recently, we have become interested in Crk and CrkL, closely related adapter proteins that regulate cell adhesion and migration in non-hematopoietic cells. To assess the function of these proteins in T cells, we generated mice that are conditionally deficient for both Crk and CrkL in the mature T-cell compartment. We find that T cells from these mice develop normally, but exhibit reduced adhesion to integrin ligands. Consistent with this, the expression of Crk and CrkL is required for efficient T-cell chemotaxis *in vitro* under conditions that involve cell adhesion. In particular, Crk/CrkL-deficient T cells are defective in their ability to cross an endothelial barrier. Biochemical studies reveal that Crk family proteins are required for activation of the integrin regulatory GTPase Rap1, and for tyrosine phosphorylation of the Rap1 GEF C3G. Defects are specific for Rap1, as activation of Rac1 is normal. Interestingly, *in vivo* studies indicate that Crk/CrkL-deficient T cells migrate efficiently to lymphoid organs, but migrate poorly to sites of inflammation. Moreover, Crk/CrkL-deficient T cells induce significantly less GVHD in a MHC mismatched murine model. The specific role of Crk proteins in this system seems to be primarily attributable to effects on T-cell migration, as effector function is unperturbed in mutant T cells. The unique and differential migratory activity of Crk/CrkL-deficient T cells has interesting therapeutic implications. Indeed, we find that T cells lacking Crk family proteins can carry out graft vs. leukemia responses without inducing GVHD. Taken together, these studies show that Crk family adaptor proteins play a key role in regulating T-cell adhesion and migration, suggesting that these proteins may represent important novel therapeutic targets.

Presenter: Hulett, Tyler

Automated Quantification of Immune Infiltrates in Human Prostate Lesions

Tyler Hulett, Carlo Bifulco, Monika Sassen, Surbhi Puri, Bernard A. Fox

Earle A. Chiles Research Institute, Providence Cancer Center; Oregon Health & Science University

Automated quantification of immune infiltrates by digital pathology - or 'immunoscore' - may soon play a role in cancer diagnostics because at least in the case of colon cancer: high levels of tumor infiltrating cytotoxic CD8+ T cells correlate with disease-free survival much better than traditional TNM scoring. Others have proposed the same could be true for radical prostatectomy patients - the most common intervention for men with prostate tumors - but published results remain inconclusive. More work is needed to understand if certain immune infiltrates correlate with different outcomes for men with prostate cancer. We are using digital pathology to begin to answer whether a difference in infiltrating immune cells - lymphoid and myeloid - will improve prediction of disease recurrence following radical prostatectomy and follow-up immunotherapies. After surgery, we created tissue microarrays from the formalin-fixed prostate tissue of 24 prostate cancer patients and 23 patients with benign lesions. Cancer patients ranged from ages 48 to 76. Institutional review board approval was obtained, and procedures were followed in accord with the ethical standards established by the Helsinki Declaration of 1975. Nineteen had Gleason scores ≥ 8 . These samples were objectively analyzed by immunostaining on a Ventana Benchmark autostainer, imaging on a Leica SCN400 whole slide scanner, and infiltrate quantification with Definiens Tissue Studio. Results demonstrate a significant increase in the count of CD68+ myeloid cell infiltrates in carcinoma versus benign lesions per tissue area ($P=0.0016$). No significant difference was observed between benign and cancerous lesions for levels of CD3+ cells ($P=0.317$). However, dramatically high levels of infiltrating CD4+ and CD8+ T lymphocytes were observed in a few outlier patients with prostate cancer. Additionally, this method will be used to quantify immune infiltrates in samples from men enrolled in our prostate cancer immunotherapy trials to determine whether pre-existing infiltrates can predict changes in PSA doubling time and survival outcomes following prostate immunotherapies. Support: Chiles Foundation, Robert W. and Elise Franz, Lynn and Jack Loacker, Wes and Nancy Lematta, the Providence Medical Foundation, and the ARCS Foundation - Portland Chapter.

Presenter: Kachura, Melissa

A Toll-like receptor 9 ligand nanoparticle formulation for prophylactic vaccination against *Bacillus anthracis* (anthrax) enhances in vivo induction of interferon and chemoattractant-associated genes

Melissa Kachura, C Hickie, SA Kell, C Calacsan, R Kiwan, R Milley, G Ott, H Kanzler, JD Campbell, RL Coffman
Dynavax Technologies

CpG-containing oligodeoxynucleotide sequences (CpG-ODN) bind TLR9 and are strong activators of innate immunity and highly effective adjuvants. To explore enhancing the potency CpG-ODN adjuvants, we have developed a nanoparticle formulation of the CpG-ODN molecule DV230 by covalent linkage to the highly cross-linked sucrose polymer, Ficoll (DV230-Ficoll). When co-administered with recombinant protective antigen (rPA) from anthrax, DV230-Ficoll demonstrated a clear potency advantage over unconjugated DV230 in enhancing the magnitude and durability of the primary immune response in both mice and monkeys. Additionally, a single DV230-Ficoll + rPA immunization protected cynomolgus monkeys from a lethal aerosol challenge with *B. anthracis*. To investigate potential mechanisms underlying the potency advantage of DV230-Ficoll nanoparticles, we performed gene microarray analysis of injection-site muscle tissue and PBMC from cynomolgus monkeys immunized once with 1 mg DV230-Ficoll + 10 mcg rPA (n=3) or 1 mg DV230 + 10 mcg rPA (n=3), with PBS-injected contralateral muscle tissue or pre-immunization PBMC serving as controls. Compared to DV230, DV230-Ficoll immunization resulted in a greater number of genes induced (≈ 2 fold in ≈ 2 monkeys/group, 18-24 hrs post-immunization) in both injection site muscle and PBMC. In addition, for genes induced by both DV230-Ficoll and DV230, DV230-Ficoll induced higher levels of gene expression. Genes related to the interferon pathway and chemoattractants were noticeably induced in DV230-Ficoll immunized monkeys. Immunization experiments in mice confirmed this pattern of preferential induction in response to DV230-Ficoll immunization. Taken together, these preliminary data suggest that the potency advantage of DV230-Ficoll compared to unformulated DV230 in vivo may be related to enhanced activation of interferon and chemoattractant-associated genes. This project was supported with funds from NIAID/NIH/DHHS under Contract No. HHSN272200800038C. The rPA protein was provided by PharmAthene, Inc.

Presenter: Kalekar, Lokesh A.

Anergic autoreactive T cells exist in the natural peripheral immune repertoire as a repository for Foxp3+ Treg progenitors

Lokesh A. Kalekar, Gretta L. Stritesky, Kristin A. Hogquist, Marc K. Jenkins and Daniel L. Mueller

University of Minnesota - Twin Cities

Anergy, an acquired state of T cell functional unresponsiveness, offers one potential solution to the problem of peripheral self antigen presentation to the adaptive immune system. We now report on the discovery of Nt5e/CD73 and Folr4/FR4 as mRNAs over-expressed during in vivo immune tolerance induction as part of an anergy gene signature, and describe a subpopulation of functionally unresponsive Foxp3^{hi}CD44^{hi}CD73^{hi}FR4^{hi} polyclonal conventional CD4 T cells in healthy hosts that is enriched for self antigen-specific TCRs. Importantly, anergic CD73^{hi}FR4^{hi} polyclonal CD4 T cells could be shown to give rise to both destructive autoreactive T effector as well as protective Foxp3⁺ Treg cells. Thus, anergy induction not only limits self antigen-driven CD4 T effector cell responses, but also generates intermediates for Foxp3⁺ peripheral Treg differentiation.

Presenter: Kell, Sariah

Toll-like Receptor 9 Ligand-Ficoll Nanoparticles are More Potent than Free TLR9 Ligands in Inducing Lymph Node Germinal Center B and T cell Responses following Immunization with Anthrax Protective Antigen in Mice

Sariah Kell, M Kachura, C Hickie, C Calacsan, R Kiwan, R Milley, G Ott, H Kanzler, RL Coffman, JD Campbell

Dynavax Technologies, Berkeley, CA

CpG-containing oligodeoxynucleotide sequences (CpG-ODN) stimulate innate immune responses by signaling through TLR9 and are highly effective adjuvants. To explore increasing the potency of CpG-ODN adjuvants, we developed a nanoparticle formulation of the CpG-ODN molecule DV230 and tested its ability to adjuvant antibody responses to anthrax recombinant protective antigen (rPA). Covalent linkage to the sucrose polymer Ficoll significantly enhanced the ability of DV230 to adjuvant high titer antibody responses to rPA in both mice and monkeys. A single immunization of monkeys with DV230-Ficoll protected the animals against aerosol challenge with 200 LD50 of *B. anthracis*. Here, we compared the relative ability of DV230-Ficoll and DV230 to induce early germinal center responses in mice immunized with rPA. BALB/c mice were injected once in the footpads with DV230-Ficoll + rPA, DV230 + rPA or Alum + rPA and draining lymph nodes were harvested from groups of mice at 3, 5, 7, and 14 days after immunization to follow kinetics of germinal center responses. As an adjuvant for rPA, DV230-Ficoll induced an early peak (Day 5) in both lymph node follicular helper T cells (Tfh cells: CXCR5 + PD1 expressing CD4+B220- cells) and germinal center B cells (GCB cells: GL7 + Fas + PNA expressing B220+CD4- cells) which was not evident in other groups, including DV230 + rPA-immunized mice. Tfh and GCB cells continued to be found in higher numbers in DV230-Ficoll + rPA immunized mice, compared to other groups, at Days 7 and 14. Similarly, DV230-Ficoll + rPA-immunized mice demonstrated preferential T and B cell expression kinetics for the transcription factor Bcl6. These data suggest that the DV230-Ficoll adjuvant nanoparticle formulation may be more potent than un-formulated DV230 in inducing high titer protective antibody responses to rPA, in part, because of early induction of strong germinal center T and B cell responses.

Presenter: Khan, Imran S.

Enhancement of an anti-tumor immune response by transient blockade of central T cell tolerance

Imran S. Khan, Maria L. Mouchess, Meng-Lei Zhu, Bridget Conley, Kayla J. Fasano, Yafei Hou, Larry Fong, Maureen A. Su, and Mark S. Anderson

University of California, San Francisco

Thymic central tolerance is a critical process that prevents autoimmunity, but also presents a challenge to the generation of anti-tumor immune responses. Medullary thymic epithelial cells (mTECs) eliminate self-reactive T cells by displaying a diverse repertoire of tissue-specific antigens (TSAs) that are also shared by tumors. Therefore, while protecting against autoimmunity, mTECs simultaneously limit the generation of tumor-specific effector T cells by expressing tumor self-antigens. This ectopic expression of TSAs largely depends on autoimmune regulator (Aire), which is expressed in mature mTECs. Thus, therapies to deplete Aire-expressing mTECs represent an attractive strategy to increase the pool of tumor-specific effector T cells. Recent work has implicated the TNF family members RANK and RANK-Ligand (RANKL) in the development of Aire-expressing mTECs. Here, we show that in vivo RANKL blockade selectively and transiently depletes Aire and TSA expression in the thymus to create a window of defective negative selection. Further, we demonstrate that RANKL blockade can rescue melanoma-specific T cells from thymic deletion and that persistence of these tumor-specific effector T cells promoted increased host survival in response to tumor challenge. These results indicate that modulating central tolerance through RANKL can alter thymic output and potentially provide therapeutic benefit by enhancing anti-tumor immunity.

Presenter: Kim, Yong Chan

Generation and functional properties of engineered antigen-specific human T regulatory cells

Yong Chan Kim, Aihong Zhang¹, Ruth A. Ettinger², Kathleen P. Pratt¹, Ethan M. Shevach³, and David W. Scott¹

¹Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, USA, ²Puget Sound Blood Center Research Institute, Seattle, WA, USA, and ³Laboratory of Immunology, National Institute of Allergy & Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Undesirable immune responses against human protein therapeutics can be a major impediment for successful therapy. A major example is the antibody response (inhibitors) against human factor VIII (FVIII) replacement therapy in hemophilia A patients. Novel efforts are necessary to overcome this problem: we propose that T regulatory cells (Tregs) can be used to prevent and reverse inhibitor formation. Clinical applications using Tregs are now considered a next-generation cellular therapy in a variety of autoimmune and inflammatory immune disorders such as type 1 Diabetes, GVHD, and transplantation, and potentially as a treatment for antibody-mediated diseases. However, therapy with polyclonal Tregs is non-specific and a potential drawback is that it could lead to undesired infection due to general immunosuppression. Therapy with antigen-specific Tregs would be highly preferable, as these have proved more potent in animal models of autoimmune diseases. We demonstrate herein the generation and functional properties of human T regulatory cells that specifically recognize FVIII. Tregs were generated that expressed an engineered T cell receptor (TCR) obtained from an HLA-DRB1*01:01-restricted T cell clone isolated from a hemophilia subject with a high-titer inhibitor. A T effector clone was isolated by MHC (DR0101) tetramer staining of this subject's CD4 cells, followed by single-cell sorting and cloning in vitro. Nucleotide/amino acid sequences were identified to design retroviral FVIII-specific TCR constructs. To produce FVIII-specific human Foxp3+ Tregs, we transduced both expanded T effector cells and Tregs with a engineered retroviral TCR. Specific reactivity of the transduced cells bearing the designed TCR was validated by staining of the transduced T effector and Treg cells by DR0101 tetramers loaded with the same peptide as that recognized by the original effector T cell clone. Transduced effector T cells were expanded by stimulation with the expected FVIII peptide in vitro. Importantly, activation by this peptide in the presence of oligonucleotides mediated functional expansion, stabilization of FoxP3 and demethylated phenotype of TSDR in ex vivo culture. Finally, the expanded transduced Tregs suppressed effector cell proliferation and cytokine secretion when stimulated by FVIII or specific peptide, even when FVIII-specific effector T cells were present in large excess. (Supported by NIH grant RO1 HL061883-15 to DWS and unrestricted research funding from Bayer, Pfizer and CSL Behring to KPP, and the intramural program of the NIAID to EMS).

Presenter: Kovar, Marek

Protein chimera of covalently linked IL-2 to S4B6 mAb exerts similar or even higher biological activity as IL-2/S4B6 mAb immunocomplexes in vivo

Marek Kovar, Marek Kovar, Jakub Tomala, Jirina Kovarova, Martina Kabesova, Petra Votavova, Helena Chmelova, Barbora Dvorakova, Blanka Rihova

Institute of Microbiology AS CR, Prague, Czech Republic

In vivo biological activity of IL-2 can be dramatically increased by complexing IL-2 with certain anti-IL-2 mAbs. One of these mAbs is S4B6 and such immunocomplexes (henceforth IL-2/S4B6 immunocomplexes) are highly stimulatory for recently activated naive CD8⁺ T cells, memory CD8⁺ T and NK cells (CD122^{high} populations), and moderately stimulatory for Treg cells. Moreover, they were reported to have potent anti-tumor effects. Unfortunately, there are some disadvantages like possible dissociation of IL-2 from mAb or excess of either substance during preparation of these immunocomplexes. Thus, we designed and produced protein chimera consisting of IL-2 linked to light chain of anti-IL-2 mAb S4B6 through flexible oligopeptide spacer (Gly₄Ser)₃ and characterized it in terms of structure that proved to be similar to IL-2/S4B6 immunocomplexes. Moreover, in vitro and in vivo experiments showed that the protein chimera mimics IL-2/S4B6 immunocomplexes not only structurally, but also functionally as it was able to stimulate proliferation of purified and activated OT-I CD8⁺ T cells in vitro and induce vigorous expansion of purified CFSE-labeled OT-I CD8⁺ T cells activated by injection of low dose of SIINFEKL peptide in vivo. This in vivo expansion was even higher than that observed after administration of IL-2/S4B6 immunocomplexes. This work was supported by Czech Science Foundation grant P301/11/0325 and 13-12885S, by the Ministry of Education, Youth and Sports grant CZ.1.07/2.3.00/30.0003 and by Institutional Research Concept RVO 61388971.

Presenter: Krishnamurthy, Akshay

Understanding B cell dynamics during blood stage malaria infection

Akshay Krishnamurthy, Damian Mazlish, Marion Pepper

University of Washington

Malaria, caused by Plasmodium parasites, is an infection that kills almost 1 million people per year. The parasite life cycle consists of an early asymptomatic hepatocyte-residing stage and a later erythrocyte-residing stage, in which clinical symptoms develop. Antibodies play a key role in conferring clinical immunity to both of these stages and several longitudinal studies amongst individuals in endemic areas have shown that B cell mediated protection is slowly acquired over time and only in the face of constant re-exposure is humoral immunity maintained. While many of these studies employ the use of ELISA-based assays to analyze serum antibody levels, such methods do not provide information as to the type of B cells that make these antibodies and how such cells are derived. Employing an antigen based enrichment method we are able to detect Plasmodium-specific B cells responding to a blood stage specific protein, Merozoite Surface Protein 1 (MSP1). Through this method we can observe the dynamics of various B cell populations over time in response to infection. In response to blood stage infection with a well characterized mouse model of malaria, Plasmodium chabaudi chabaudi (AS), we observe at the peak of infection the emergence of MSP1-specific extrafollicular, short-lived plasmablasts (PBs), and as parasitemia begins to decline we observe the formation of later arising MSP1-specific germinal center (GC) B cells. GC-derived memory B cells are maintained over time with a majority of these cells expressing a class-switched phenotype. Surprisingly, there is also an emergence of MSP1-specific GC-independent B cells, which also form a stable memory population to levels similar to that of GC-derived memory cells. Amongst this population we find both unswitched and class switched cells. Together these data show that in response to primary infection, three heterogeneous populations of Plasmodium-specific B cells emerge with two stable memory populations maintained over time. We are currently assessing the functional contributions of each of these memory populations to protective immunity. These studies represent the first glimpse of the endogenous B cell response to Plasmodium with the hopes of better informing vaccine design to elicit the induction of optimally protective B cell responses against the Plasmodium parasite.

Presenter: Krow-Lucal, Elisabeth

Human fetal and adult monocytes have distinct transcriptional profiles and JAK/STAT responses

Elisabeth Krow-Lucal, Charles C. Kim, Trevor D. Burt, Joseph M. McCune

UCSF

Preterm birth affects one out of nine infants born in the United States and is the leading cause of long-term neurological handicap and infant mortality, accounting for 35% of all infant deaths in 2008. Although cytokines including IFN, IL-10, IL-6, IL-1, and TNF are produced in response to in utero infection and are strongly associated with the onset or prevention of preterm labor, little to nothing is known about how human fetal immune cells respond to these cytokines. Here, we demonstrate that fetal and adult CD14⁺16⁻ classical monocytes are distinct from one another, both in terms of basal transcriptional profiles and in their phosphorylation of signal transducers and activators of transcription (STATs) in response to inflammatory stimuli. Compared to adult monocytes, fetal monocytes phosphorylate both canonical and non-canonical STATs and respond more strongly to multiple cytokines (e.g., IFN, IL-6, and IL-4). We show evidence for a potential mechanism to explain these differences in STAT phosphorylation by demonstrating a higher ratio of SOCS3 to IL-6 receptor in adult monocytes, relative to fetal monocytes. In addition, IFN signaling results in up-regulation of antigen presentation and co-stimulatory machinery in adult but not fetal monocytes. These findings represent the first evidence that the immune response in primary human fetal monocytes is functionally distinct from the adult, providing a foundation for understanding how these cells respond to cytokines implicated in development, in utero infections, and in the pathogenesis of preterm labor.

Presenter: Kuan, Emma

The role of thymic stromal lymphopoietin in promoting breast tumor progression and metastasis via myeloid derived suppressor cells

Emma Kuan, Steven Ziegler

Benaroya Research Institute

Not to be placed on website.

Presenter: Kverka, Miloslav

Immunogenicity of coiled-coil based drug-free macromolecular therapeutics

Miloslav Kverka, Jonathan M. Hartley, Te-Wei Chu, Jiyuan Yang, Regina Heidchen, Jindrich Kopecek

Institute of Microbiology AS CR, 14220 Prague, Czech Republic; and University of Utah, Salt Lake City, UT 84112, USA

We analyzed the immunogenicity of coiled-coil based drug-free macromolecular therapeutics that were effective in an animal model of human B-cell lymphoma. Therapeutics were prepared by attachment of anti-human CD20 Fab' to the synthetic pentaheptad peptide that forms coiled-coil with a complementary peptide covalently bonded to linear copolymer N-(2-hydroxypropyl)methacrylamide. The synthetic peptides contain either L or D amino acids. The therapeutics and their components were cultivated with murine macrophages RAW264.7. Cell viability and surface expression of CD40 and CD127 was measured by flow cytometry, production of cytokines by ELISA and production of nitrite by Griess assay. Most compounds prepared from the original antibody activated macrophages and reduced their viability. Pre-mixture based on L amino acids (MIX L) induced higher expression of CD127, higher production of TNF-A and lower production of IL-6 than pre-mixture based on D amino acids (MIX D). The reduction of viability, expression of CD40 and production of nitrite was similar in both pre-mixtures. To analyze immunogenicity, immuno-competent BALB/c mice were injected intravenously with MIX L or MIX D and serum IgM and IgG levels were subsequently analyzed by ELISA. The antibody response was low and antibodies had low avidity, except for the anti-Fab conjugate IgM response, which showed both high titer and high avidity. MIX D induces slightly lower antibody response with antibodies of lower avidity than MIX L. The use of D instead of L amino acids in synthetic peptides induces a different type of macrophage response in vitro and lower antibody response in vivo. Supported by NIH grant GM095606 (to JK) and by the Ministry of Education, Youth and Sports of the Czech Republic project CZ.1.07/2.3.00/30.0003.

Presenter: Lau, Laura

Exploring Viral Antagonists of the Interferon Stimulatory DNA (ISD) pathway

Laura Lau, Sarah Wilson, Jason G. Smith, Daniel B. Stetson

University of Washington

A key aspect of antiviral immunity is the induction of IFNs to mediate the effective clearance of a viral infection. The interferon stimulatory DNA (ISD) pathway detects cytosolic DNA in mammalian cells and initiates the activation of a robust antiviral type I interferon (IFN) response. Downstream of this pathway is an ER resident adapter, stimulator of IFN genes (STING), which is known to play a part in mediating the signaling of upstream DNA-binding sensor(s) to IFN activation. In order to avoid detection and prevent the induction of type I IFNs, viruses have evolved antagonists to the host response. Our preliminary data demonstrates that the ISD pathway is highly active in primary cells but is completely absent from all transformed and tumor cell lines we have tested. This leads us to consider whether there is a connection between oncogenic transformation and inhibition of the ISD pathway. To this end, we have defined DNA virus-encoded antagonists that potently and specifically inhibit the ISD pathway at a proximal step upstream of STING activation. Understanding the mechanism of ISD pathway blockade has important implications for the evolution of interactions between DNA viruses and their hosts.

Presenter: Lee, Hyeon-Woo

TMEM126A, a CD137 ligand binding protein, is involved in TLR4-mediated maturation in myeloid cells

HYEON-WOO LEE,

Institute of Oral Biology, School of Dentistry, Kyung Hee University, Seoul, South Korea, 130-701

Our recent study showed that a novel transmembrane protein 126A (TMEM126A) binds to CD137 ligand (CD137L, 4-1BBL) and couples with its reverse signals in myeloid cells (Cell Signal., 2012, 24, 2222-). Here, we present data showing TMEM126A relays TLR4 signals inducing myeloid cellular maturation. ICAM-1, CD86 CD40 and MHC II expression up-regulated by LPS treatment were diminished in TMEM126A knock-down myeloid cells compared with control cells. However, LPS-induced up-regulation of IL-6 and TNF- α expression was intact in these cells. These data suggest that TMEM126A is involved in TLR4 signals up-regulating expression of genes for antigen presenting to T cells without affecting those for pro-inflammatory cytokines.

Presenter: Lin, Joseph

Hysteresis of ERK Signaling in T Cells is Regulated by its Subcellular Localization

Joseph Lin, Hannah Fields

Sonoma State University

Studies examining the induction and duration of T cell activation have led to the conclusion that prolonged signaling events are required to ultimately initiate T cell effector functions. However, the important question of how cells "remember" past and ongoing signaling events remains unanswered. To investigate this type of hysteretic behavior, we monitored the phosphorylation state of the MAP kinase, ERK, upon TCR stimulation, followed by induced termination of signaling events using the Src kinase inhibitor PP2. We found that the rate of ERK deactivation occurred rapidly when the cells had been activated for only a short period of time, whereas ERK deactivation occurred more slowly when ERK had been activated for prolonged periods of time. This hysteretic behavior correlated with the localization of ERK. At early time points following activation, phosphorylated ERK displayed enrichment in the nucleus; at later time points, however, ERK remained phosphorylated, but its localization became predominantly cytoplasmic. When ERK was forcibly sequestered in the cytoplasm, initiation of ERK activation was not altered, however the deactivation kinetics was substantially slowed. Therefore, we conclude that the localization of ERK can have profound effects on the kinetics of its deactivation, in part allowing the cell to display hysteretic behavior following T cell activation.

Presenter: Livingston, Justin

Apoptotic and Necrotic Cancer Cells Differentially Affect Macrophage Aggressiveness

Justin Livingston, Gajendra Shrestha, Eugene Lee, Evita Weagel, Kim O'Neill

Brigham Young University

Macrophages are heterogeneous cells that have a variety of phenotypes, most notably M1 and M2. Macrophages play an important role in cancer progression and development. The majority of tumor associated macrophages have been identified as M2 macrophages. Based on our understanding of tumor anatomy and macrophage behavior, necrotic and apoptotic cancer cells may affect macrophage polarization differently. We hypothesized that macrophages exposed to necrotic cancer cells would have higher aggressiveness, as measured by engulfment, as compared to apoptotic or control cancer cells. We used human macrophages to investigate the effects of the tumor microenvironment on macrophage phenotype. We performed a macrophage engulfment assay using fluorescent beads by co-culturing macrophages with either necrotic, apoptotic, or Raji cells. We measured macrophage engulfment rates of the beads using flow cytometry. The percentage of the macrophage population that engulfed beads for necrotic co-culture was 64.2% and 70.1% higher than apoptotic and control co-cultures respectively. We also saw that the percent of engulfing macrophages from necrotic co-culture that had engulfed at least 3 beads was on average 44.9% and 45.42% higher than macrophages from apoptotic and control co-cultures respectively. Our preliminary results show that macrophages co-cultured with necrotic cancer cells are more aggressive than ones co-cultured with control or apoptotic cancer cells. This indicates that necrotic and apoptotic cancer cells may induce different macrophage phenotypes. We believe the high aggressiveness of macrophages co-cultured with necrotic cancer cells can be attributed to an M1 phenotype while the mild aggressiveness of the macrophages co-cultured with apoptotic cancer cells indicates an M2 phenotype. However, further work is needed to confirm these preliminary results.

Presenter: Lucas, Beth

CCRL1 in the thymus

Beth Lucas, Graham Anderson, Antal Rot

University of Birmingham, UK

Thymus colonisation and thymocyte positioning are mediated by interactions of CCR7 and CCR9 and their ligands CCL19/CCL21 and CCL25, respectively. These chemokines also interact with the atypical receptor CCRL1, which is expressed within the thymus and may impact on chemokine availability and function. Given the conflicting literature regarding the functional involvement of CCRL1 in intrathymic T cell development, we have utilized CCRL1-GFP reporter mice and CCRL1 deficient mice to perform a systematic analysis of the expression of CCRL1 and its potential function in the adult and embryonic thymus. We show CCRL1 expression within three distinct microenvironments of the thymus: the cortex, the corticomedullary junction (CMJ) and the subcapsular zone (SCZ.) Within the cortex, CCRL1 is expressed predominantly by immature MHC-II^{low}CD40⁻ cortical epithelial cells, identifying heterogeneity within this compartment. CCRL1 expression at the SCZ is by non-epithelial, podoplanin positive cells. Interestingly, CCRL1 is expressed by non-epithelial, non-endothelial cells, which surround the A-SMA⁺ pericytes of vessels at the CMJ - the route for progenitor cell entry and mature thymocyte egress from the thymus. Analysis of the embryonic CCRL1 KO thymus compared to littermate controls revealed increased frequencies of early thymic progenitors, suggestive of a role for CCRL1 in controlling the recruitment of progenitors to the thymus during later embryonic stages. However, analysis of adult CCRL1 KO mice showed no differences in thymus size and cellularity, and no major perturbations in intrathymic T-cell development. Overall, our findings argue against a major role for CCRL1 in normal thymus development and function.

Presenter: Lucas, Carrie L.

Activating mutations in p110[h] cause human immunodeficiency and reveal a prominent role of PI3K in CD8+ effector T cell differentiation and senescence

Carrie L. Lucas, Michael J. Lenardo

Laboratory of Immunology, NIAID, NIH

The p110 δ subunit of phosphatidylinositol-3-kinase (PI3K) is selectively expressed in leukocytes and is critical for lymphocyte biology. We have identified fourteen patients from seven families who were heterozygous for one of three different germline, gain-of-function mutations in PIK3CD (which encodes p110 δ) and suffered from sinopulmonary infections, lymphadenopathy, nodular lymphoid hyperplasia, and viremia due to cytomegalovirus (CMV) and/or Epstein-Barr virus (EBV). Strikingly, hyperactivation of PI3K signaling caused significant skewing of peripheral blood CD8+ T cells toward an effector phenotype. In vitro, T cells from patients exhibited increased phosphorylation of the kinase Akt and exaggerated effector characteristics, including increased granzyme B, T-bet, and IFN- γ expression as well as augmented degranulation. Mechanistically, T cell blasts harboring gain-of-function mutations in p110 δ had constitutively hyperactive mTORC1 and enhanced glucose uptake. These changes promoted marked terminal effector cell differentiation and senescence of CD8+ T cells. Notably, treatment of patients with rapamycin to inhibit mTOR activity in vivo partially restored the abundance of naive T cells, largely 'rescued' the in vitro T cell defects, and greatly improved the clinical course.

Presenter: Manz, Boryana

Negative regulation of SFK by Dok1

Boryana Manz, Ying Xim Tan, Arthur Weiss

UCSF

Multiple regulatory mechanisms finetune strength and duration of the immune response to pathogens and cancer. Src family kinases (SFK) mediate early antigen receptor signaling and are tightly regulated spatially and temporally. The potent SFK negative regulator, Csk, can be recruited in an inducible manner by the adaptor family Dok, but the role of this negative regulation in primary T lymphocytes is poorly understood. Using a synergy of biochemical, genetic and imaging approaches, we aim to characterize the role of basal and inducible Dok1 mediated Csk localization and SFK activity. We provide evidence that Dok1 regulates SFK in human cell lines and primary murine T lymphocytes and will discuss possible mechanisms of regulation.

Presenter: Martin, Christopher

Clean up on IL-7: maintenance of IL-7 homeostasis by the cytokine sink

Christopher Martin, Darina Spasova, Kwesi Frimpong-Boateng, Charles Surh

La Jolla Institute for Allergy and Immunology

IL-7 availability determines the size and composition of the resting T cell pool. The crucial role of IL-7 represents a point for therapeutic intervention to control T cell numbers and naïveté. However, the mechanisms that determine IL-7 homeostasis remain unclear. Using novel mouse models and the IL-7-specific proliferative response of mature T cells as a direct measure of IL-7 availability in vivo, we demonstrate that radioresistant cells are the peripheral source of IL-7 for both CD4+ and CD8+ mature T cells. Hematopoietic lineage cells, although apparently unimportant as a source of IL-7, are primarily responsible for limiting its availability through their expression of IL-7R. Within the IL-7R+ hematopoietic population, we implicate innate lymphoid cells as potent regulators of IL-7 levels in primary and secondary lymphoid tissue. These results directly support the consumption model of IL-7 homeostasis wherein IL-7 is constitutively produced by stromal cells, but is maintained in a limited supply by hematopoietic cells through receptor-mediated degradation. Collectively, these data indicate that a formidable IL-7 cytokine sink exists in vivo and should be considered in the design of IL-7 therapy.

Presenter: Messenheimer, David

Autophagosome-enriched tumor vaccine combined with costimulation primes effector T cells in a spontaneous murine tumor model, but in combination with costimulation and checkpoint blockade cannot impact tumor burden.

David Messenheimer, Christopher G Twitty, Michael Afentoulis, Shawn M Jensen, Bernard A Fox

Earle A. Chiles Research Institute, Providence Cancer Center; Oregon Health & Science University

Immunotherapies in cancer have recently shown great potential, with combination therapies proving to be particularly effective. Combined PD-1 and CTLA-4 checkpoint blockade has shown substantial effects in clinical trials with almost 50% of late stage melanoma patients experiencing tumor regression. However over half of the treated patients failed to benefit from this therapy and our preclinical experience suggests that these patients' tumors were "poorly immunogenic". Therefore we reasoned that vaccines which prime tumor-specific T cells will play an important role in treating these tumors which do not respond to checkpoint blockade. Recently we developed an autophagosome-enriched tumor vaccine, by blocking the proteasome and lysosomal degradation, which accumulates short-lived and defective proteins (SLiPs and DRiPs). Based on the work of Yewdell and colleagues we hypothesize these SLiPs and DriPs represent tumor antigens that the host is not tolerant of. This vaccine combined with costimulation provides significant therapeutic efficacy in three transplantable mammary cancer models (4T1 and C57MG, and MMTV-derived FAT). Given reports of minimal levels of T cell infiltrate in spontaneous MMTV-PyMT-derived tumors we anticipated that the MMTV model may well represent hard to treat, "immune-score"-negative human tumors, which show scant immune infiltration. Accordingly we tested the therapeutic efficacy of our vaccine in combination with blockade of PD-1 and CTLA-4 in the MMTV-PyMT tumor model hypothesizing that priming of a more diverse repertoire of effector cells would be necessary for therapeutic effect. Effector T cells generated ex vivo from treated tumor-bearing and non-tumor-bearing mice that received a vaccine and costimulation were capable of secreting tumor-specific IFN- γ ($p < 0.001$). We also saw significant increases in proliferating Ki67+CD8+ T cells and CD62L-CD44hiCD4+ effector T cells ($p < 0.05$) in the vaccinated lymph nodes one week after vaccination in both tumor-bearing and non-tumor-bearing mice compared to antibody alone-treated groups. However, only non-tumor-bearing mice exhibited significant increases of these effector T cell populations in the spleen. Despite apparently successful tumor-specific effector T cell priming, vaccination with or without combination therapy failed to impact tumor growth, number of tumor foci, lung metastases, or survival in MMTV-PyMT mice in two independent experiments ($n=8$ /group). We hypothesize that although our vaccine was capable of priming effector T cells, these cells were suppressed by non-PD-1, non-CTLA-4 mechanisms or did not successfully traffic to the tumor. Consequently we are further characterizing the tumor microenvironment and alterations to the immune system in the host and will explore other therapies capable of impacting mechanisms of tumor immune suppression to combine with our vaccine. Support: Chiles Foundation, Robert W. and Elise Franz, Lynn and Jack Loacker, Wes and Nancy Lematta, and the Providence Medical Foundation.

Presenter: Miller, Michelle

Lentivirus-activated CD4+CD25+ T regulatory cells convert CD4+CD25- T helper cells into fully functional regulatory cells.

Michelle Miller, Christopher Petty, Jonathan Fogle

North Carolina State University College of Veterinary Medicine, Raleigh, NC 27607, USA

We and others have previously reported that CD4+CD25+ Treg cells are activated during in vivo infection and suppress CD4+CD25- T helper cells by a membrane-bound TGF β (mTGF β) dependent mechanism. We have also reported that a novel protein known as Glycoprotein A Repeats Predominant (GARP) anchors mTGF β to the Treg cell surface and facilitates suppressor activity. Recently, we have described that these immunosuppressive GARP+TGF β + Treg cells expand during the course of feline immunodeficiency virus (FIV) infection. As Treg cells are anergic and generally exhibit poor proliferative ability, we asked how Treg homeostasis is maintained during lentiviral infection. Here we provide evidence that Treg cells from FIV+ cats express GARP and mTGF β and convert T helper (Th) cells into phenotypic and functional induced Treg (iTreg) cells. Flow cytometry and PCR of the converted Th cells confirms the upregulation of Treg specific markers at both the protein and mRNA level. Suppression assays further demonstrate Th cells converted to iTreg cells are able to suppress IL2 production and proliferation of other activated T cells in vitro. Th to iTreg conversion was abrogated by anti-TGF β or anti-GARP treatment of Treg cells or by anti-TGF β R2 treatment of Th cells prior to coculture, suggesting that Treg cell recruitment from the Th pool is mediated by TGF β /TGF β R2 signaling and that GARP-anchored TGF signaling plays an important role in this process. We believe this to be the first report describing the conversion of Th cells to iTregs by lentivirus-activated Treg cells. These findings suggest Th to iTreg conversion is likely one of the mechanisms that contributes to the maintenance of virus reservoirs, progressive Th cell immunosuppression, and the development of immunodeficiency, all of which are central to the pathogenesis of AIDS lentivirus infections.

Presenter: Mills Ko, Emily

Chemokine CXCL10 expressed in astrocytes drives T cell infiltration in experimental autoimmune encephalomyelitis

Emily Mills Ko, Monica Moreno, Fuzheng Guo, Laird Meirs, Athena Soulika, Travis Burns, David Pleasure

UC Davis

Multiple Sclerosis (MS) is an inflammatory neurological disorder, characterized by demyelination, and axonal degeneration eventually leading to severe neurological deficits. The chemokine CXCL10 is chemotactic for T cells and contributes to lymphocyte migration to inflammatory sites. Our lab and studies by others show that CXCL10 is induced primarily in astrocytes in the murine MS model, experimental autoimmune encephalomyelitis (EAE). We have created a mouse in which *cxcl10* is deleted specifically in astrocytes (GFAP (Glial fibrillary acidic protein)-Cre-CXCL10^{fl/fl}) to investigate the effect of CXCL10 on EAE-mediated immune cell infiltration of the CNS in a cell-specific manner. Mice receive myelin oligodendrocyte glycoprotein (MOG) EAE induction, with non-EAE, and non-GFAP-Cre controls run in parallel. The mice were monitored daily for clinical severity, and sacrificed before, during and after onset of neurological deficits. The immune responses in the CNS were examined via flow cytometry. Brain and spinal cord tissues were examined immunohistologically for evidences of axonopathy, demyelination, and for inflammatory infiltrate composition. We have found that deletion of CXCL10 in astrocytes during EAE results in reduced severity of clinical disease course. When we analyzed the composition of infiltrating cells, we found fewer CD4 T cells, and an increase in infiltrating macrophages. This change in infiltrating cells may contribute to reduced inflammation and improved clinical outcomes. We did not observe any axon protection; however myelin was protected at peak disease. These findings will contribute to potential therapies targeting CXCL10 for the treatment of MS.

Presenter: Molinder, Karen

Developing a diagnostic assay for internal abscesses caused by *Corynebacterium pseudotuberculosis* in horses

Karen Molinder, Daniel Crowley, Roberta Pollock

Occidental College

Corynebacterium pseudotuberculosis is an intracellular, facultative anaerobic bacterium that infects horses predominantly in the southwestern United States but with increasing frequency in other regions. In horses there are distinct manifestations of the disease, commonly known as pigeon fever. The most common manifestation is an external infection characterized by abscesses in the pectoral or ventral muscles. Horses can also suffer from internal infections where abscesses form on vital organs. While diagnosing external infections is straightforward, quickly and reliably identifying an internal infection in horses has been quite challenging. Until recently, clinicians have used the synergistic hemolysis inhibition (SHI) assay to diagnose internal infections by measuring titer levels of antibodies against the major bacterial exotoxin. Data from our lab has shown that these antibody levels alone cannot differentiate between the different manifestations of the disease. Our recent findings suggest that a Western Blot assay to determine which *Corynebacterium pseudotuberculosis* proteins are detected by antibodies from infected horses may be a better diagnostic tool. Preliminary evidence has shown that horses with different manifestations of the disease have different patterns of *C. pseudotuberculosis* protein recognition. We plan to perform a single blind study to further test if horses can be accurately diagnosed with our western blot assay.

Presenter: Morris, Gerald

Naive alloreactive and autoreactive T cell repertoires are a consequence of secondary TCR[α] rearrangements designed to maximize positive selection efficiency

Gerald Morris, Peggy P. Ni, Chyi-Song Hsieh, Paul M. Allen

University of California, San Diego

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Presenter: Myers, Darienne R.

A missense variant in Rasgrp1 increases CD44 expression on na \tilde{A} ^{-ve} T cells and drives mTOR-dependent accumulation of PD1⁺ Helios⁺ T cells and autoantibodies

Darienne R. Myers, Stephen R. Daley, Kristen M. Coakley, Daniel Hu, Katrina L. Randall, Craig N. Jenne, Andre Limnander, Christopher C. Goodnow, Jeroen P. Roose

University of California, San Francisco

Rasgrp1^{Anaef} is a new mouse missense variant with an ENU-mutated EF hand of the ras guanine nucleotide exchange factor Rasgrp1. Rasgrp1^{Anaef} peripheral T cells uniformly upregulate CD44 in a cell intrinsic manner, a portion of these CD4⁺ CD44^{high} cells adopt an activated, PD1⁺Helios⁺ state, and anti-nuclear antibodies are detected in the blood of Anaef mice. Despite impaired Rasgrp1^{Anaef}-Ras-ERK signaling in vitro, thymocyte development is relatively intact in Anaef mice. We subsequently identified CD44 as a sensitive reporter for mTOR-S6 activity in T cells and found that peripheral CD4⁺ Rasgrp1^{Anaef} T cells have elevated tonic mTOR-S6 signaling. This aberrant mTOR activity is essential for the observed T cell phenotype and autoimmune pathology, as crossing Rasgrp1^{Anaef} mice to a reduction-in-mTOR-function mouse, mTOR^{chino}, resolves the T cell and autoimmune features. We are currently interested in determining the biochemical mechanisms of Rasgrp1-mTOR signaling as well as the immunological impacts on helper T cell differentiation and function. As missense variants are a major source of human genetic variation and variants are often correlated with autoimmune disease, we see Rasgrp1^{Anaef} as an exciting tool to understand how aberrant signaling can drive T cell dysregulation in the context of autoimmunity.

Presenter: Nichols, Randilea

Role of Caspase-7 in NLRC4-dependent control of Legionella pneumophila

Randilea Nichols, Mary F. Fontana, Jakob von Moltke, and Russell E. Vance

University of California, Berkeley

Legionella pneumophila is thought to secrete flagellin through its type four secretion system, leading to activation of the NAIP5/NLRC4 inflammasome. Caspase-1 is the canonical caspase recruited to the inflammasome, and has been shown to be required for processing of interleukins-1B and -18 into their mature forms. In vitro experiments have shown Caspase-1 can also cleave Caspase-7. In fact, previous work has suggested that in the context of L. pneumophila infection, Casp7 macrophages phenocopy Casp1 macrophages, implying that Caspase-7 is the primary effector downstream of NAIP5/NLRC4 and Caspase-1. In contrast to this previous work, we observe that Casp7^{-/-} macrophages, singly deficient in Caspase-7, are not defective for control of L. pneumophila replication. However, consistent with previous reports, we also show that Nlrc4^{-/-} macrophages are more permissive to L. pneumophila than Casp1^{-/-} macrophages, indicating there is another pathway dependent on NAIP5/NLRC4 but independent of Caspase-1. Our work demonstrates this alternative pathway involves Caspase-7, acting parallel to (rather than downstream of) Caspase-1. Casp1^{-/-} Casp7^{-/-} macrophages are as permissive to L. pneumophila replication as Nlrc4 macrophages; moreover, Casp1^{-/-} Casp7^{-/-} macrophages are as resistant to pyroptosis as Nlrc4^{-/-} macrophages, whereas Casp1^{-/-} and Casp7^{-/-} macrophages still exhibit NLRC4-dependent pyroptosis. We find that tumor necrosis factor alpha is important for stimulating this parallel Caspase-7 inflammasome. Taken together, our results identify Caspase-7 as an important Caspase-1-independent effector of the NLRC4 inflammasome.

Presenter: Nowakowska, Dominika

The role of PTPN22 in the homeostasis and function of Treg.

Dominika Nowakowska, Peilin Zheng, Stephan Kissler

Joslin Diabetes Centre

Regulatory T cells (Treg) are critical to the maintenance of immune tolerance, partly by controlling the unwanted activation of effector T cells (Teff) and thereby enhancing the resolution of autoimmune inflammation. An allelic variant of protein tyrosine phosphatase nonreceptor 22 (PTPN22) has been associated with multiple autoimmune diseases such as diabetes and rheumatoid arthritis. PTPN22 is expressed in all hematopoietic cells including Treg. Here we use doxycycline-induced knock down (KD) of PTPN22 to examine its role in the homeostasis and function of Treg. Previous work in our laboratory has shown that PTPN22 KD mice have increased percentages of Foxp3⁺ cells in the periphery but not in thymus which could imply increased Treg conversion *in vivo* or affected homeostasis of thymically-derived Treg (nTreg) in the periphery. Preliminary data indicate that *in vivo* conversion of conventional Foxp3⁻ T cells to Foxp3⁺ T cells (induced Treg, iTreg) is unaffected however the stability of Foxp3 expression seems to be higher in PTPN22 KD iTreg. We did not observe changes in the Foxp3 stability or cell proliferation in nTreg transferred into lymphopenic host. However, upon systemic Treg depletion with anti-CD25, PTPN22 KD Treg replenished peripheral Treg pool significantly faster compared to WT Treg. Ongoing work is using Affymetrix gene expression assay to examine how silencing PTPN22 affects Treg homeostasis and function.

Presenter: O'Gorman, William

The split-virus influenza vaccine activates Fc[g] receptors instead of Toll-like receptors

*William O'Gorman, Huang Huang, Yu-Ling Wei, Kara Davis, Michael Leipold, Sean Bendall,
Brian Kidd, Cornelia Dekker, Holden Maecker, Yueh-Hsiu Chien, and Mark Davis*

Stanford University

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Presenter: Ohtsuka, Shizuka

Dual effects of ERK pathway on the clonal expansion of antigen specific CD4+ T cells

Shizuka Ohtsuka, Shuhei Ogawa, Ryo Abe

Division of Immunobiology, Research Institute for biomedical Sciences, Tokyo University of Science

Antigenic stimulation is one of the critical factors controlling T cell clonal expansion. However, the molecular mechanism that regulates T cell proliferation in response to a wide range of antigens is not fully understood. Therefore, in this study we investigated the signaling pathways regulating T cell proliferation by using an OVAp-specific CD4+ T cell clone whose maximal proliferation is observed at a low antigenic peptide dose, and whose proliferation is diminished at a high peptide dose. Furthermore, high concentration of IL-2 was observed at a high dose. The number of apoptotic cells and the expression of Fas/FasL were induced at both low and high dose, and increased in a dose-dependent manner. However, proliferation of T cells exposed to the high dose was not restored by treatment with anti-FasL Ab or caspase inhibitor. In addition, with the high dose, we noted accumulation of T cells in S phase and lower populations of those in G2/M phase, that is, entry into G2/M phase was inhibited at a high dose of peptide. Taken together, these data suggested that the suppression of cell growth at a high dose was primarily induced by cell cycle arrest rather than by apoptosis caused by AICD. Since several signaling molecules, such as ERK and Akt, were also activated in a dose-dependent manner, we investigated the relationships between these molecules in terms of activation and proliferation by using specific inhibitors. Both inhibitors suppressed the production of IL-2. But, interestingly, MEK inhibitor rescued S phase arrest and increased proliferation at a high peptide dose, while it decreased the proliferation of T cells at a low dose. In contrast, Akt inhibitor suppressed proliferation at each peptide dose. These data indicated that the ERK signaling pathway has both positive and negative effects on effector T cell clonal expansion depending on the strength of antigenic stimulation. We are now investigating how the ERK pathway regulates cell cycle arrest.

Presenter: Osokine, Ivan

Type I interferon inhibits de novo CD4 Th1 cell development during an established persistent virus infection.

Ivan Osokine, Cameron R. Cunningham, Douglas H. Yamada, Laura M. Snell1, Elizabeth Wilson, Heidi Elsaesser, Juan Carlos de la Torre and David G. Brooks

University of California, Los Angeles

CD4 T cells are central to orchestrate, sustain and potentially regenerate antiviral immunity throughout persistent viral infections. Although the evolving immune environment during persistent infection reshapes established CD4 T cell responses, the fate of naïve CD4 T cells primed after viral persistence has been established is unclear. We demonstrate that in marked contrast to the onset of infection, virus-specific CD4 T cells primed during an established persistent infection fail to develop Th1 responses, to efficiently traffic to peripheral tissues and rapidly and almost exclusively differentiate into T follicular helper cells. Th1 cells are associated with enhanced control of multiple persistent viral infections, and the inability to add new Th1 cells could have a dramatic effect on the control of virus infection by failing to sustain CD8 T cell responses as well as other immune subsets (e.g. dendritic cells and macrophages). The failure of de novo Th1 generation and tissue homing was mediated by chronic type I interferon (IFN-I) production and was effectively restored by blocking IFN-I signaling. We demonstrate that IFN-I acts via a virus-specific CD4 T cell-independent mechanism during T cell priming to repress Th1 immunity. Thus, chronic IFN-I signaling prevents de novo antiviral CD4 Th1 immunity during an established persistent viral infection.

Presenter: Pearce, Hayden

MAGE-specific T cells are elevated in the peripheral blood of testicular germ cell tumour patients

Hayden Pearce, Shalini Chaudhri, Paul Hutton, Richard Viney, Prashant Patel, Emilio Porfiri, Paul Moss

University of Birmingham

The expression of cancer testis antigens (CTAGs) is normally restricted to spermatogenic cells of the testis but is also present in many cancers including testicular germ cell tumours (TGCTs). CTA-specific T cell responses have been identified in patients with various solid tumours, and here we identified CTA-specific T cells in TGCT patients. MAGEA family-specific T cells were detected in 21/49 patients with a magnitude of up to 0.149% of total peripheral blood mononuclear cells. Responses to multiple MAGEA antigens were frequently detected in seminoma patients irrespective of tumour stage. Conversely, NSGCTT patients only developed responses towards MAGEA3, which were strongly associated with disease progression. Longitudinal analysis revealed that the magnitude of MAGE-specific immune responses diminished over time by up to 95%, which correlated with an assumed reduction in tumour antigen load. MAGE-specific CD8 T cells demonstrated cytotoxic potential against peptide loaded LCLs and endogenously presented MAGE antigen. Of interest, T cells infiltrating testicular tumour lesions were antigen experienced, recently activated, oligoclonal populations; many of which expressed the inhibitory molecules, TIM-3 and PD-1. Our data suggests MAGE-specific T cells undergo clonal expansion and maintain functional capacity following recognition of tumour-derived cognate antigen, which leads to an increased frequency in peripheral blood.

Presenter: Pelletier, Adam-Nicolas

NOD mice reveal novel genetic loci linked to a multi-step regulation of NK cell maturation

Adam-Nicolas Pelletier, Fanny Guimont-Desrochers, Sylvie Lesage

Université de Montréal

Natural Killer cells (NK) constitute potent innate lymphocyte effector cells that exhibit a major role in tumour immunosurveillance and viral clearance. Moreover, mature NK (mNK) cells have been shown to regulate components of both the innate and adaptive branches of the immune system via a variety of mechanisms. Their functional maturation, which follows a 4-step developmental program according to the expression of CD11b and CD27, separates NK cells into distinct subsets with specific phenotypic, functional and migratory properties. The genetic determinants involved in the regulation of NK cell maturation remain uncharacterized. We observed a block in splenic NK cell maturation in the NOD mouse strain in comparison to the C57BL/6 strain, suggesting a genetic control of the phenotype. In order to identify the factors involved in the regulation of NK cell functional maturation, we performed a linkage analysis on (C57BL/6 x NOD) mouse strain F2 intercross. We identified several major loci on chromosomes 2, 4, 7, 10, 11 and 18 associated with different stages of NK cell maturation. These results not only highlight the multigenic regulation of NK cell functional maturation, but suggest each maturation step is tightly regulated by distinct factors. A subsequent in silico analysis will provide a restricted list of candidate genes by using deep-sequencing of the C57BL/6 and NOD strain genomes to identify polymorphic genes.

Presenter: Pestal, Kathleen

The Regulation of Nucleic Acid Sensing by ADAR1

Kathleen Pestal, Daniel B Stetson

University of Washington

Adenosine deaminases that act on RNAs (ADARs) have been shown to deaminate adenosines in pre-mRNA, noncoding RNAs and viral RNAs to create inosines. Inosine is translated as guanosine, which can lead to altered codons and differential splicing, resulting in multiple protein isoforms from the same gene. Additionally, inosine binds to uracil with less affinity as compared to adenosine, leading to instability in the structure of the target RNA. More than 130 mutations in ADAR1 have been associated with Dyschromatosis Symmetrica Hereditaria, a discoloration of the skin, and more recently, an additional 9 mutations are associated with Aicardi-Goutieres Syndrome, a genetically determined inflammatory disorder of the brain and skin. Adar1^{-/-} mice die in utero at embryonic day 12 and have an aberrant type I interferon (IFN) response that may contribute to embryonic lethality. We attempted to rescue the embryonic lethality by crossing Adar1^{+/-} mice to mice deficient in key molecules involved in type I IFN signaling, including: STING, a signaling molecule associated with the antiviral response to intracellular DNA and MAVS, which is required for the cell-intrinsic response to RNA. Embryos harvested at day 11.5 were analyzed for their expression of several known interferon stimulatory genes (ISGs) by quantitative RT PCR. While both the ADAR1^{-/-} and the ADAR1^{-/-}STING^{-/-} embryos show elevated ISG expression, the ADAR1^{-/-}MAVS^{-/-} embryos have a clear reduction in interferon signature. Additionally, two ADAR1^{-/-}MAVS^{-/-} mice were rescued to birth. This data suggests that ADAR1 regulates the sensing of RNA.

Presenter: Ping-Chih, Ho

CD40L- and IFN-mediated Signaling Is Required for BRAF Inhibitor-mediated Anti-tumor Immunity

Ho Ping-Chih, Katrina Meeth, Bhaskar Srivastava, Yao-Chen Tsui, Marcus Bosenberg, Susan Kaech

Yale University

Not to be placed on website.

Presenter: Priatel, John

SAP is Essential for B Cell-Priming of Antigen-Specific CD8 T Cell Responses.

John Priatel, Yu-Hsuan Huang, Kevin Tsai, Kenneth W. Harder, Rusung Tan

University of British Columbia

Not to be placed on website.

Presenter: Proekt, Irina

Defects in Lyn and Aire pathways cooperate to promote autoimmune uveitis.

Irina Proekt, Marion Jeanne, Kayla Fasano, Douglas B. Gould, Mark S. Anderson, Anthony L. DeFranco

University of California, San Francisco

Studies of genetic factors associated with human autoimmune disease point to a multigenic origin of autoimmune susceptibility. However, little is known about how defects in different immune tolerance checkpoints cooperate to result in full-blown disease. To address this, we chose to investigate whether genetic alterations in two pathways implicated in immune tolerance might synergize to lead to autoimmunity in mice. The intracellular protein tyrosine kinase Lyn is critical for inhibitory receptor function in both B cells and myeloid cells. Lyn-deficient mice develop lupus-like systemic autoimmune disease but not organ-specific autoimmunity. In contrast, Autoimmune Regulator (Aire) promotes the expression of tissue-specific antigens by thymic epithelial cells and T-cell negative selection. Aire-deficient mice develop multiorgan autoimmunity, including autoimmune uveitis that has been linked to absence of thymic expression of retina-specific protein, interphotoreceptor retinoid binding protein (IRBP), and expansion of IRBP-specific CD4 T cells. A knockin mouse model with a single mutant allele of Aire encoding a G228W mutation (Aire^{GW/+}) has low but detectable expression of IRBP and does not develop eye disease on a C57BL/6 genetic background. To determine whether the lack of Lyn inhibitory pathways can cooperate with a partial defect in T cell central tolerance to lead to organ-specific autoimmunity, we created a double mutant Aire^{GW/+} Lyn^{-/-} mouse. Remarkably, over 50% of double mutant mice developed severe uveitis that was not detected in parental genotypes and was accompanied by a rise in anti-IRBP antibodies and an expansion of IRBP-specific CD4 T cells. Interestingly, although Lyn^{-/-} mice do not develop retinal autoimmunity, funduscopy revealed small abnormalities in the retinas of these mice. Flow cytometric analysis of Lyn^{-/-} retinal cell populations showed an expansion of CD45^{hi} CD11b⁺ myeloid cells, including increased numbers of activated F4/80⁺ macrophages. Furthermore, CD11b⁺ cells were detected in the subretinal space of Lyn^{-/-} but not wt mice. These results suggest that Lyn-deficient retinal myeloid cells may promote uveitis induction by autoreactive T cells that have escaped negative selection due to partial loss of function of Aire.

Presenter: Racine, Ronny

**CD44 Expression Induces Calcium Influx Modifying EGR-1 Expression and Proliferation
in Acute Lymphocytic Leukemia T Cells**

Ronny Racine, Jessica Hall, Adnan Dibas, Mark Mummert

University of North Texas Health Science Center

CD44 is a cell surface glycoprotein that serves as the major receptor for hyaluronan, aiding in trafficking and adhesion of immune cells. CD44 also serves as a recruitment platform for signaling molecules and has been shown to regulate proliferation. We have shown that CD44 expression in Jurkat T cells causes a decrease in proliferation. In our current study, we have observed that CD44 expression greatly increases the influx of calcium from extracellular sources. Calcium influx is necessary for the proliferation of T cells, but CD44 expressing Jurkat cells show a disrupted calcium homeostasis. Through use of calcium channel inhibitors we have shown that Jurkat T cells rely on calcium release activated calcium channels for influx. We have observed that CD44 induced excess calcium influx negatively regulates early growth response protein 1 expression, which is responsible for the decrease in proliferation. Our findings show for the first time that CD44 can influence the calcium signaling of leukemic T cells, impacting their proliferation and potentially making a less aggressive cancer cell.

Presenter: Raghu, Harini

Coagulation Factor XIII Deficiency Diminishes Inflammatory Arthritis In Mice Through Fibrinogen-dependent and -Independent Mechanisms

Harini Raghu, Carolina Cruz, Cheryl L. Rewerts, Malinda D. Frederick, Sherry Thornton and Matthew J. Flick

Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease characterized, in part, by pathological coagulation system activity within affected joints (e.g., fibrin deposition along articular cartilage/inflamed synovium). The transglutaminase, factor XIII (fXIII), plays a crucial role in coagulation through stabilizing fibrin clots by catalyzing the formation of γ -glutamyl- γ -lysyl crosslinks. In addition, fXIII has been shown to promote multiple processes important in arthritis pathogenesis such as fibroblast proliferation, angiogenesis, monocyte/macrophage migration and activation. To test the hypothesis that fXIII promotes arthritis pathogenesis by driving local inflammatory and tissue degradative/remodeling events, we challenged mice deficient in the catalytic A subunit of fXIII (i.e., fXIIIA^{-/-}) collagen-induced arthritis (CIA). Comparative macroscopic analyses of CIA-challenged animals revealed that arthritis incidence and severity were significantly diminished in fXIIIA^{-/-} mice compared to their fXIIIA-sufficient littermate controls. fXIIIA^{-/-} mice also displayed quantitatively diminished knee-joint pathology (e.g., inflammatory cell infiltration, synovial hyperplasia, pannus, and cartilage/bone erosion). Consistent with a reduction in disease severity, local expression of effector cytokines (e.g., IL1 β , IL6), chemokines (e.g., RANKL) and the osteoclast-specific bone resorptive enzyme, TRAP, were significantly reduced in fXIIIA^{-/-} mice compared to controls. Strikingly, analysis of unchallenged fXIIIA^{-/-} mice indicated a significant diminution in the local expression of osteo-immunologic effectors RANKL and TRAP, suggesting that fXIIIA may direct activities of the cartilage/bone-remodeling unit. Importantly, the observed changes were independent of the primary fXIII target, fibrin(ogen), as no differences in the local expression of osteologic markers were observed in unchallenged fibrinogen-deficient mice compared to fibrinogen-sufficient controls. In complementary studies, pharmacologic inhibition of transglutaminase activity using cystamine, revealed a similar diminution in the local expression of RANKL and TRAP in naive DBA/1 mice relative to naive vehicle-treated animals. DBA/1 mice pre-treated with cystamine prior to the CIA-challenge developed attenuated inflammatory joint disease compared to CIA-challenged vehicle-treated mice. Collectively, these findings indicate that elimination of fXIII transglutaminase activity significantly reduces arthritis severity and reduces cartilage and bone destruction, in part, through mechanisms linked to osteoblast-osteoclast signaling/activation. Further defining the role of fXIII in local destructive events during arthritis pathogenesis may highlight novel therapeutic strategies for inflammatory arthropathies such as RA.

Presenter: Rawlings, Jason

Mechanism of activation induced chromatin decondensation in peripheral T cells

Jason Rawlings, Megan Lee, Taylor Mitchell, Jenna Meredith, Kellie Bingham

Furman University

Chromatin condensation is a requirement for proper T cell development and maintenance of the naive, quiescent state. T cells remain in this quiescent state until they participate in an immune response to a foreign antigen during which antigen-specific T cells gain the ability to proliferate in response to interleukin-2 (IL-2). Previous studies showed that activation via the T cell receptor (TCR) results in the decondensation of chromatin, allowing STAT5 target genes essential for proliferation to be expressed in response to subsequent IL-2 stimulation. To ensure clonal proliferation of only those T cells specific for the presented antigen, the chromatin of other naive T cells must remain condensed preventing both activation and proliferation. Activation via the TCR results in the hydrolysis of phosphoinositol biphosphate (PIP₂) into inositol triphosphate (IP₃) and diacylglycerol (DAG). IP₃ binds to the IP₃ receptor on the endoplasmic reticulum resulting in the release of calcium from intracellular stores while DAG activates protein kinase C (PKC) leading to the activation of multiple signaling pathways including Ras/MAPK. Previous work in our lab showed that both signaling pathways are capable of decondensing chromatin. Here, we investigate the role that the IP₃ and PKC pathways play in decondensing chromatin. We show that extracellular calcium is not necessary for chromatin decondensation; however, intracellular calcium is both sufficient and required for chromatin decondensation. Furthermore, we show that activation of PKC is also required. Interestingly, while both pathways can decondense chromatin, only the DAG pathway is capable of permitting the expression of STAT5 target genes. This evidence suggests that there may be two distinct domains of chromatin that must be decondensed for proper T cell activation and proliferation.

Presenter: Resop, Rachel

S1P-R1 is increased on mature thymocyte subsets after HIV-1 infection of the human thymus

Rachel Resop, Joshua Craft, Marc Douaisi, Christel Uittenbogaart

University of California, Los Angeles

Lack of T cell regeneration in HIV infection despite successful antiretroviral therapy is likely due in part to an impact on egress of naive T cells from the thymus to the periphery. However, there are few data available elucidating the impact of HIV on these processes. We studied the effect of HIV on the receptors to Sphingosine-1-phosphate (S1P), a chemotactic sphingolipid. In the mouse it has been shown that S1P plays an important role in the egress of mature thymocytes to peripheral blood. We have shown that human thymocytes migrate to S1P and that FTY720 inhibits migration by functioning as an analogue to S1P, thereby downregulating S1P receptor 1 (S1P-R1), which is important for thymocyte egress. During T cell development S1P-R1 expression is significantly increased at the mRNA and protein level in the most mature CD3hiCD69- thymocyte subset about to exit the thymus as mature naive T cells. We have also shown that in thymocytes S1P-R1 responds to S1P exposure with increased Akt phosphorylation, internalization of the receptor upon binding, and downregulation of S1P-R1 mRNA. Here we present data on the dynamics of S1P-R1 expression in the human thymus during HIV infection, which has thus not been described. Two separate series of NSG mice implanted with human thymus/liver (thy/liv) grafts and infected with both CXCR4- and CCR5-tropic HIV-1 were used to analyze the effect of HIV on S1P-R1 in the human thymus implant. Persistent infection and immune activation were verified by demonstrating that IFN-alpha secondary genes, MxA and ISG15, are upregulated. Surprisingly, our results show that S1P-R1 as well as its transcriptional regulator, Kruppel-Like Factor 2 (KLF2) were both significantly upregulated in mature infected thymocytes at several time points after HIV infection. S1P-R1 function after HIV infection of the human thymus implants was investigated in vitro and ex vivo by directly measuring Akt signaling induced by S1P/S1P-R1 binding. Our findings indicate that S1P-R1 signaling is not impaired in infected thymocytes, which is in contrast to published data in other cell types that point to impaired S1P-R1 response in HIV infection. Moreover, we found that CFSE-labeled CD34+ progenitors developed into mature thymocytes in the human thy/liv implant of infected NSG mice and that a subset expressed S1P-R1 and still migrated to the periphery. Our main objective is to understand how diseases that cause inflammation like infections, cancer and autoimmune diseases, affect migration of lymphocytes from thymus and peripheral lymphoid tissues by changing S1P and S1PR expression. Future experiments with HIV will focus on additional measures to examine whether S1P-R1 is fully functional on thymocytes after HIV infection. We will additionally perform in vitro co-transfection assays with a KLF2 reporter construct and HIV tat to determine if the underlying cause of S1P-R1 upregulation is due to HIV activation of the transcriptional regulator KLF2. If S1P-R1 remains upregulated and fully functional at various time points post HIV-1 infection, this discovery may offer insight into T cell reconstitution mechanisms during infection as well as provide a potential alternate immunotherapy for patients.

Presenter: Rizzuto, Gabrielle

Innate immune response to *Listeria monocytogenes* infection at the maternal-fetal interface

Gabrielle Rizzuto, Anna Bakardjiev

UCSF

Pregnancy is a unique immunological situation because the fetus is not genetically identical to the mother. The placenta plays an important role in protecting the fetus from attack by the maternal immune system while at the same time protects the fetus from pathogens. In contrast to other organs (liver, spleen), the placental immune response to pathogens is not well understood. We want to characterize the differences in innate immune responses in the placenta versus these other organs. To do so, we employ *Listeria monocytogenes* (LM), a clinically relevant placenta pathogen in humans, in order to probe immune defense at this site. Our hypothesis is that maternal uterine stromal cells, placental macrophages, and recruited inflammatory myeloid cells defend the placenta and fetus against pathogens. Before embryo implantation, remodeling of the uterine lining (endometrium) occurs with phenotypic and functional stromal cell changes. This process is called decidualization and the pregnancy endometrium is termed decidua. Infection spreads from maternal blood stream to the decidua prior to infecting the fetal part of the placenta and fetus. Thus, our host model systems include a combination of primary human decidua and endometrium organ cultures, human endometrial stromal cell lines and primary human endometrial stromal cells that can be decidualized in culture, as well as a mouse model of artificial decidua formation. Our results show variable growth over time of LM in human decidua but not endometrium cultures. Preliminary histology shows that bacteria localize within human decidual stromal cells, and that macrophages do not aggregate near foci of bacteria. However, intracellular growth and cell-to-cell spread of LM is restricted in monolayer cultured human decidual stromal cells. Results from our in vivo mouse infection model show that the decidua is resistant to infection, but once seeded LM grows and spreads rapidly. We observe marked macrophage recruitment and infiltration to the infected mouse decidua but, curiously, not to the particular anatomic sites where bacteria are concentrated. Ongoing and future experiments are aimed at determining (1) how decidual stromal cells restrict growth and cell-to-cell spread of *Listeria* and (2) whether or not macrophages aid in defense within the decidua.

Presenter: Roberts, Lydia

Revealing sub-dominant immune signaling pathways following *Francisella tularensis* infection

Lydia Roberts, Hannah Ledvina, Jeff Frelinger
University of Arizona

Francisella tularensis is a facultative, intracellular coccobacillus and the causative agent of tularemia. *F. tularensis* utilizes a variety of strategies to evade the host's immune response. One evasion mechanism involves the suppression of a pro-inflammatory cytokine response in the lung following intranasal inoculation with wild-type live vaccine strain (LVS). We have identified a *F. tularensis* mutant, LVS *clpB*, which fails to inhibit early pro-inflammatory cytokine and chemokine production in the lung. TLR2 has been identified as the dominant innate sensor of *Francisella* as TLR2-deficient macrophages fail to produce pro-inflammatory cytokines after infection with several strains of *Francisella*. Using LVS *clpB* as a tool, we identified three classes of cytokines/chemokines that differ in their dependence on TLR2 signaling for production. IL-1 α , IL-1 β , IL-2, IL-17, MIP-1 α , and TNF- α production depended on TLR2 signaling while GM-CSF, IFN and VEGF production was completely independent of TLR2 signaling. IL-6, IL-12, IP-10, KC, and MIG production was partially dependent on TLR2 signaling. Although TLR2 KO mice are able to clear an LVS *clpB* infection, bacterial clearance is delayed compared to wild-type mice. The delayed clearance of LVS *clpB* in TLR2 KO mice correlates with decreased IFN production by CD4⁺ and CD8⁺ T cells in the lung. Together, our data indicate that while the dominant innate sensor is TLR2, other innate pathways also sense *Francisella* and are capable of initiating an immune response that successfully mediates bacterial clearance.

Presenter: Roose, Jeroen

A Rasgrp1^{Anaef} mutation increases naive T-cell CD44 expression and drives mTOR-dependent accumulation of Helios⁺ CD44^{hi} CD4⁺ T cells and autoantibodies

Jeroen Roose, Goodnow and Roose groups, Chris Goodnow and Jeroen Roose

UCSF

Missense variants are a major source of human genetic variation. We identified and analyzed a new mouse missense variant, Rasgrp1^{Anaef}, with an ENU-mutated EF hand in the Rasgrp1 Ras guanine nucleotide exchange factor. Despite reduced Rasgrp1-Ras-ERK activation in vitro, thymocyte selection in Rasgrp1^{Anaef} is mostly normal in vivo, although CD44 is overexpressed on naive thymocytes and T cells in a T-cell-autonomous manner. A portion of these CD4⁺ CD44^{hi} T cells gradually take on a Helios⁺ PD-1⁺ quasi-helper T cell phenotype, which is dependent on B cells, and results in the production of anti-nuclear autoantibodies. We subsequently identified CD44 expression as a sensitive reporter of tonic mTOR-S6 kinase signaling through a novel mouse strain, chino, with a reduction-of-function mutation in Mtor. Elevated tonic mTOR-S6 signaling occurs in Rasgrp1^{Anaef} naive CD4⁺ T cells. CD44 expression, CD4⁺ T cell subset ratios and serum autoantibodies all returned to normal in Rasgrp1^{Anaef} Mtor^{chino} double-mutant mice, demonstrating that increased mTOR activity is essential for the Rasgrp1^{Anaef} T cell dysregulation. Extrapolating from our recent insights in the structure of Rasgrp1, Rasgrp1^{Anaef} appears a faulty “on” and faulty “off” variant and a useful model to study autoimmunity without lymphopenia. Rasgrp1 mutation increases naive T-cell CD44 expression and drives mTOR-dependent accumulation of Helios⁺ CD44^{hi} CD4⁺ T cells and autoantibodies. Stephen R. Daley, Kristen M. Coakley, Daniel Hu, Katrina L. Randall, Craig N. Jenne, Andre Limnander, Darienne R. Myers, Noelle K. Polakos, Anselm Enders, Carla Roots, Bhavani Balakishnan, Lisa A. Miosge, Geoff Sjollem, Edward M Bertram, Matthew A Field, Yunli Shao, T. Daniel Andrews, Belinda Whittle, S. Whitney Barnes, John R. Walker, Jason G. Cyster, Christopher C. Goodnow, and Jeroen P. Roose. eLIFE, 2013 December. The Structural Basis for Autoinhibition of the Ras-specific exchange factor RasGRP1. Jeffrey S. Iwig, Yvonne Vercoulen, Rahul Das, Tiago Barros, Andre Limnander, Yan Che, Jeffrey G. Pelton, David E. Wemmer, Jeroen P. Roose# and John Kuriyan#. eLIFE, 2013 July.

Presenter: Sampson, James

Galectin-8 promotes regulatory T cell differentiation and inhibits proinflammatory Th17 cells

James Sampson, Amol Suryawanshi, Wei-Sheng Chen, Noorjahan Panjwani
Tufts University

Regulatory T cells (Tregs) play a critical role in limiting inflammation and preventing immunopathology in many contexts. For instance, Tregs prevent development of autoimmune retinal uveitis, and ameliorate active disease by inhibiting Th1- and Th17-mediated inflammation. Uveitis affects approximately 1 in 5000 people in the United States, where it is responsible for 10-20% of all cases of blindness. Here, we report that a B-galactoside-binding protein, galectin-8 (gal-8), promotes Treg differentiation and induces apoptosis of Th17 cells. We demonstrate that gal-8 binds IL-2R (CD25) and TGFB receptor on CD4+ T cells, thereby sustaining STAT5 phosphorylation and enhancing Smad3 phosphorylation; these phenomena correlate with increased Treg differentiation. Furthermore, Tregs polarized in the presence of gal-8 have elevated expression of the immunosuppressive cytokine IL-10 and the suppressive co-receptor CTLA4. Furthermore, we find that gal-8-polarized Tregs are functional, and able to suppress effector T cell proliferation in vitro. Gal-8 is a good candidate for treatment of autoimmune uveitis since it induces apoptosis of Th17 cells and promotes Treg differentiation. Gal-8 treatment may represent an alternative to corticosteroids and immunosuppressive drugs that leave the patient immunocompetent.

Presenter: Sauer, Karsten

Metabolic Inhibition by Inositol-tetrakisphosphate Delays Thymocyte γ -Selection and Renders it Notch-Dependent

Karsten Sauer, Luise Sternberg, Yina H. Huang, Stephanie Rigaud, Lyn'Al Nosaka, Claire Conche, Sabine Siegemund

The Scripps Research Institute

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Presenter: Sauer, Karsten

Soluble IP4 Limits NK Cell Effector Functions by Controlling PI3K Signaling

Karsten Sauer, Eugene Park, Sabine Siegemund, Anthony French, Joseph Wahle, Luise Sternberg, Stephanie Rigaud, Helena Jonsson, Wayne Yokoyama, Yina Huang

The Scripps Research Institute

Not to be placed on website.

Presenter: Schmidt, Amanda M.

Diacylglycerol Kinase [z] Limits the Generation of Natural Regulatory T Cells

*Amanda M. Schmidt, Tao Zou, Rohan P. Joshi, Theresa M. Leichner, Matthew A. Pimentel,
Connie L. Sommers, Taku Kambayashi*

University of Pennsylvania

Natural regulatory T (nTreg) cells are important for maintaining peripheral tolerance, and are thought to develop from thymocytes receiving strong T cell receptor (TCR)-mediated signals in the thymus. However, the specific signaling pathways from the TCR leading to nTreg development are not fully understood. TCR engagement leads to the activation of phospholipase C γ 1, which generates the lipid second messenger diacylglycerol (DAG) from phosphatidylinositol-4,5-bisphosphate. Here, we used mice that lack the Z isoform of DAG kinase (DGKZ), which metabolizes DAG to terminate its signaling, to enhance TCR-mediated signaling and identify critical signaling events in nTreg cell development. Loss of DGKZ resulted in a significant increase in thymic CD25⁺Foxp3⁺CD4⁺ nTreg cell precursors and Foxp3⁺CD4⁺ nTreg cells in a cell-autonomous manner. DGKZ-deficient T cells exhibited increased nuclear translocation of the nuclear factor B subunit c-Rel, as well as enhanced extracellular signal-regulated kinase (ERK) phosphorylation in response to TCR stimulation, suggesting that these downstream pathways may contribute to nTreg cell development. Indeed, diminution of c-Rel or blockade of ERK phosphorylation abrogated the increased nTreg generation in DGKZ-deficient thymocytes. Additionally, the amount of ERK phosphorylation could be directly correlated with TCR-induced Foxp3 induction in immature thymocytes, and nTreg development was augmented in mice bearing selectively enhanced ERK activation. Together, these data suggest that DGKZ regulates the development of nTreg cells by limiting the extent of activation of the ERK and c-Rel signaling pathways.

Presenter: Schmiel, Shirdi

A role for ecto-5'-nucleotidase (CD73) in promoting anergy

Shirdi Schmiel, Daniel Mueller

University of Minnesota

Anergy is a peripheral tolerance mechanism required to control self-reactive lymphocytes that escape central tolerance during their development. Regulatory T cells (Tregs) are required for the induction of CD4 T cell anergy; however, the mechanisms responsible for Treg-mediated anergy have yet to be identified. CD73, a cell surface ecto-5'-nucleotidase (Nt5e) expressed on Foxp3⁺ Tregs may be important for the maintenance of immune tolerance to glucose-6-phosphate isomerase (GPI), a self antigen targeted by T and B cells in a mouse model of autoimmune arthritis. Recently, we reported that CD73 is also highly expressed on anergic GPI/MHCII-specific KRN CD4⁺ T cells. Therefore, we have been interested in determining the role of CD73 in promoting and maintaining anergy. Adoptive transfer experiments now show that host expression of CD73 is necessary to promote CD73 up-regulation and anergy induction within the GPI-reactive KRN CD4⁺ T cells following their recognition of self antigen. Consequently, wildtype KRN T cells caused arthritis following their adoptive transfer to GPI/MHCII-expressing Nt5e-deficient, but not Nt5e-sufficient hosts. Ultimately, understanding the mechanisms involved in the induction, maintenance, and breakdown of anergy will help us develop therapeutic strategies to restore immune homeostasis in patients suffering from autoimmune disorders.

Presenter: Schuster, Cornelia

CLEC16A modulates thymic epithelial cell autophagy and impacts T cell selection

*Cornelia Schuster, Kay D. Gerold, Kilian Schober, Lilli Probst, Mi-Jeong Kim, Thomas Serwold,
Stephan Kissler*

Joslin Diabetes Center, Harvard Medical School

CLEC16A variation has been associated with multiple immune-mediated diseases, including type 1 diabetes, multiple sclerosis, systemic lupus erythematosus, celiac disease, Crohn's disease, Addison's disease, primary biliary cirrhosis, rheumatoid arthritis, juvenile idiopathic arthritis and alopecia areata. Despite strong genetic evidence implicating CLEC16A in autoimmunity, this gene's contribution to disease remains uncertain because its biochemical and cellular functions have not been characterized. Here we show that CLEC16A modifies disease risk by modulating thymic selection, owing to a role in thymic epithelial cell (TEC) autophagy. We generated *Clec16a* knock-down (KD) mice in the nonobese diabetic (NOD) model for type 1 diabetes and found that *Clec16a* silencing protected against autoimmunity. Disease protection was attributable to T cell hyporeactivity that was secondary to changes in TEC stimuli that drive thymocyte selection. Negative selection was increased and the frequency of recently selected (CD69+) thymocytes was diminished in *Clec16a* KD mice. *Clec16a* silencing affected TEC autophagy in vivo and disruption of autophagy by knockdown of either *Clec16a* or the established autophagy gene *Atg5* in a TEC-derived cell line affected thymocyte differentiation in vitro in a manner consistent with changes observed in *Clec16a* KD mice. Our data demonstrate a role for *Clec16a* in TEC autophagy and show that T cell selection and reactivity are impacted by *Clec16a* variation in thymic epithelium. These findings provide a functional link between human CLEC16A variation and the immune dysregulation that underlies the risk of autoimmunity.

Presenter: Shrestha, Gajendra

Metastatic Breast Cancer Strongly Polarizes Macrophages to an M2 Phenotype

Gajendra Shrestha, Evita Weigel, Justin Livingston, Larry St. Clair, Richard Robison, Kim O'Neill

Brigham Young University

Macrophages are one of the most important cells of the innate immune system and are phylogenetically conserved in all multicellular organisms. They play an important role in cancer progression and development. Depending upon their cytokine profile, macrophages have been classified as M1 and M2. M1 macrophages inhibit cell proliferation and are aggressive phagocytes while M2 macrophages promote cell proliferation and tissue repair. Various studies have shown that the microenvironment has the ability to polarize M1 macrophages to an M2 phenotype to aid tumor development. One of the most dangerous aspects of cancer development is metastasis. Metastasis, the spread of a tumor or cancer to distant parts of the body from its original site, generates serious concern for cancer treatment because most cancer deaths are caused by metastasis. However, all tumors are not metastatic in nature. It has been shown that highly metastatic tumors acquire alterations in more genes than non-metastatic tumors, and various genes are differentially expressed between metastatic and non-metastatic tumors. Hence we have hypothesized that metastatic tumors create a microenvironment that polarizes M1 macrophages to M2. To test our hypothesis we co-cultured monocyte-derived human macrophages with two different breast cancer cell lines - MCF-7 (non-metastatic) and MDA-MB-231 (highly metastatic) and compared the macrophage aggressiveness as measured by engulfment of fluorescent beads. We also measured the cytokine profiles of the macrophages to support our hypothesis on macrophage polarization. Our results showed that macrophages co-cultured with MDA-MB-231 had more radical suppression of engulfment than macrophages co-cultured with MCF-7. The percentage of engulfment by macrophages co-cultured with MDA-MB-231 was 18.97% while with MCF-7 had 33.5%. The engulfment rate in control samples (macrophage polarized with LPS in the absence of cancer cells) was 51.83%. We also found that lower engulfment rates correlate with an M2 cytokine profile and higher engulfment rates correlate with an M1 cytokine profile. Our preliminary results support our hypothesis that metastatic tumors polarize the M1 macrophages to M2 for cancer proliferation and overall survival. Further in vitro as well as in vivo research is needed to understand more of the signals that cancer cells give to their environment to prevent macrophage engulfment and to help maintain an M2 phenotype.

Presenter: Slichter, Chloe

Cytokine-Mediated Activation of Mucosal Associated Invariant T Cells

Chloe Slichter, Brenda Seymour, Andrew McDavid, Greg Finak, Raphael Gottardo, Martin Prlic
Fred Hutchinson Cancer Research Center

Mucosal-Associated Invariant T (MAIT) cells respond directly to bacterial vitamin B metabolites via their invariant T cell receptor (TCR) when presented by the non-classical MHC I molecule MR1. In contrast to the established role MAIT cells have during bacterial infections, their contribution to the immune response in the absence of TCR stimuli has been minimally investigated. The presence of MAIT cells in mucosal tissue, which is often the initial site of pathogen exposure, their abundance in the periphery (1-8% of T cells) and liver (20-40% of T cells), and the constitutive expression of pro-inflammatory cytokine receptors make MAIT cells one of the first responders to infection prior to the onset of typical adaptive immunity. We examined the ability of MAIT cells to respond to inflammatory cues in the absence of bacterial metabolites and found that inflammation is sufficient to induce MAIT cell activation including acquisition of cytotoxicity and secretion of pro-inflammatory cytokines and chemokines. Additionally, transcriptional analysis of MAIT cells circulating in the blood and residing in the mucosal tissue revealed unique gene expression profiles compared to conventional CD8+ T cells and NK cells. These data indicate a differential role for MAIT cells depending on the site and context of infection. Our data suggest that MAIT cells play a much broader role in the immune system than previously anticipated and indicate important functions of MAIT cells beyond their currently established role as anti-bacterial effector cells.

Presenter: Snell, Laura M.

Polarized Th1 Responses Fail To Be Sustained In the Midst of Persistent Viral Infection

Laura M. Snell, Ivan Osokine, David G. Brooks

Department of Microbiology, Immunology and Molecular Genetics and the UCLA AIDS Institute, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California

Not to be placed on website.

Presenter: Staron, Matthew

FoxO1 directly regulates the expression of PD-1 and sustains viral specific CD8+ T cell responses during chronic infection

Matthew Staron, Simon Gray, Ian Parish, Guoliang Cui, Ming Li, WK Eddie Ip, and Susan Kaech

Yale University

Understanding how signaling and transcriptional pathways are integrated to regulate CD8 T cell (CTL) exhaustion during chronic infection are fundamental to the development of therapies to treat chronic diseases such as HIV, HSV, and HCV. Of these, the PI3K/AKT/mTOR signaling pathway is of significant interest because it not only integrates responses to both TCR and cytokines to regulate the differentiation of effector CTL, but also provides effector CTL with the appropriate anabolic metabolism to carry out their anti-viral functions. The inhibitory receptor, PD-1, has been shown to suppress T cell receptor (TCR) signaling; however, the relevant signaling pathway(s) downstream of PD-1 in regulating CTL exhaustion during chronic infection remains unexplored. Here we show that, during chronic viral infection, therapeutic blockade of the PD-1 signaling pathway that restores viral specific CTL responses is coupled to an increase in PI3K/AKT/mTOR activity, and is sensitive to the mTOR inhibitor rapamycin. Moreover, we find that chronic antigen stimulation of exhausted CTL greatly impairs their ability to activate the PI3K/AKT/mTOR signaling pathway in response to TCR ligation, resulting in enhanced nuclear retention of the transcription factor, FoxO1. FoxO1 expression was critical to sustain viral-specific CTL responses and to control chronic infection. Lastly, we demonstrate that FoxO1 was both necessary and sufficient to regulate the expression of the inhibitory receptor PD-1 in vitro and in vivo. These findings highlight a novel role for AKT-FoxO1 pathway in the regulation of exhaustion CTL responses during chronic infection.

Presenter: Steck, Ryan

Pharmacoregulation of inflammation in monocyte-derived macrophages

Ryan Steck, Spencer Hill, Richard Robison, Kim O'Neill

Brigham Young University

Caffeine's mechanism of action in immune cells has been an issue of debate for some time. There is a significant body of evidence suggesting that many of the physiological effects of dietary caffeine are mediated by antagonism of adenosine receptors (Mandel 2002). The activation of adenosine receptors on immune cells generally leads to the suppression of pro-inflammatory cytokines (Ohta and Sitkovsky 2001). Therefore, if the primary effect of caffeine on macrophages was to antagonize adenosine receptors, we would expect caffeine to stimulate pro-inflammatory responses. However, caffeine suppresses TNF-alpha production in whole-blood macrophages (Horrigan, Kelly et al. 2004). In order to investigate this suppressive effect, we studied the inflammatory profile of macrophages after exposure to caffeine. Using a phagocytic assay and gene expression assays, we sought to further elucidate caffeine's mechanism of action at both physiological and supraphysiological concentrations. Caffeine's suppressive effect on phagocytosis at physiological levels was blocked by pre-treatment with a PKA inhibitor. At supraphysiological levels of caffeine, cells treated with PKA inhibitor showed an increase in phagocytosis over controls. IL-10 production increased, and TNF-alpha production decreased with increasing concentrations of caffeine. Macrophages are highly involved in regulation of inflammation, and dysregulation has been implicated in a number of diseases, including cancer. These results suggest further potential for the therapeutic applications of caffeine and its analogs for the control of inflammation.

Presenter: Stone, Erica

Control of T follicular helper cell differentiation by FOXO1

*Erica Stone, Marion Pepper, Carol D. Katayama, Yann M. Kerdiles, Yin C. Lin, Edward Yang,
Ananda W. Goldrath, Shane Crotty, Stephen M. Hedrick*

UC San Diego

T follicular helper (Tfh) cells constitute an essential cell type in the induction of high affinity, class-switched antibodies. The differentiation of Tfh cells is a multi-step process that depends upon ICOS and the activation of PI3K leading to the expression of key Tfh genes. We report that ICOS signaling inactivates FOXO1 and loss of FOXO1 allows for generation of Tfh cells and with reduced dependence on ICOSL. FOXO1 regulates Tfh differentiation through a broad program of gene expression exemplified by its negative regulation of Bcl6. Final differentiation to germinal center Tfh cells (GC-Tfh) was conversely FOXO1 dependent as the Foxo1^{-/-} GC-Tfh population was substantially reduced, and this correlated with reduced ICOS expression. These results demonstrate that ICOS inactivation of FOXO1 initiates a Tfh transcriptional program that is completed in a FOXO1-dependent manner.

Presenter: Sumida, Megan

A Murine Model for Equine *Corynebacterium pseudotuberculosis* Vaccine Development

Megan Sumida, Kristi Geiger

Occidental College

Pigeon Fever is an equine disease caused by the bacterium *Corynebacterium pseudotuberculosis*. It has three manifestations: external abscesses, internal abscesses, and ulcerative lymphangitis. Our main goals are to understand the equine immune response against this bacterium and to use a mouse model to create a successful vaccine. C57Bl/6 mice are used to study the effects of a strong Th1 immune response; these are compared to BALB/c mice that exhibit a strong Th2 immune response. Mice were immunized with formalin-inactivated components of *C. pseudotuberculosis* or with phospholipase D (PLD), the major secreted exotoxin. Mice were challenged intradermally with live bacteria at a previously determined lethal dosage. ELISA analysis of sera samples show both BALB/c and C57Bl/6 mice make a high IgG anti-PLD when vaccinated with PLD. Both strains of mice make a high IgG response against cell lysate when vaccinated with cell lysate or cell debris. In general, the BALB/c strain produces a higher antibody response than the C57Bl/6 strain. BALB/c mice that showed a high IgG response to PLD had a higher survival rate than mice with lower antibody responses. Cell debris showed the best protective immune response in terms of subject survival for both strains of mice. Overall, BALB/c mice displayed higher survival rates than C57Bl/6 mice, especially with the PLD vaccine. Monoclonal antibodies to *C. pseudotuberculosis* PLD and cell lysate proteins have been generated and are being used to identify proteins recognized by infected horses and challenged mice. Such proteins are good potential vaccine components. The identified proteins will be cloned and the recombinant proteins tested for effectiveness as protective vaccine components.

Presenter: Tan, Ying Xim

Csk kinase activity tunes the threshold for T cell receptor signaling

Ying Xim Tan, Chao Zhang, Kevan M Shokat, Arthur Weiss

University of California, San Francisco

Not to be placed on website.

Presenter: Tenthorey, Jeannette

Molecular basis for specific recognition of bacterial ligands by NAIP/NLRC4 inflammasomes

Jeannette Tenthorey, Eric Kofoed, Matt Daugherty, Harmit Malik, Russell Vance
UC, Berkeley

NAIP/NLRC4 inflammasomes assemble in response to the presence of bacterial products, including flagellin and components of the type III secretion system (T3SS), in the host cell cytosol. Ligand specificity is dictated by the NAIP component, of which multiple highly related paralogs are expressed in mice. For example, NAIP5 detects monomeric flagellin, whereas NAIP2 mediates the response to inner rod proteins of the T3SS. Using a reconstituted NAIP/NLRC4 inflammasome in 293T cells, we generated NAIP2-NAIP5 chimeric proteins in order to map the ligand specificity-determining domain of NAIPs. Surprisingly, ligand specificity was not mediated by the previously described leucine-rich repeat (LRR) domain of NAIPs. Instead, an uncharacterized internal domain between the nucleotide-binding domain (NBD) and LRR was both necessary and sufficient to dictate NAIP ligand specificity. Interestingly, we find that this ligand-specificity domain has evolved under positive selection in both rodents and primates, consistent with the direct association of these domains with rapidly evolving bacterial ligands. Ligand binding then licenses NAIP proteins to co-oligomerize with NLRC4, and an assessment of the stoichiometry of the assembled complex indicates that on average, more than one NAIP protomer is present in a single assembled NAIP/NLRC4 inflammasome. Furthermore, this second protomer is as likely to be an alternate NAIP paralog, provided that both cognate ligands are present during assembly. These data suggest that ligand binding is strictly required for incorporation of each NAIP protomer into the inflammasome. Together, the above data provide insight into the mechanism by which NAIP/NLRC4 inflammasomes assemble in response to the cytosolic detection of cognate bacterial ligands.

Presenter: Thelen, Tennille

The Role of IL-25 in the Initiation of a Type-2 Inflammatory Response

Tennille Thelen, Steven F. Ziegler

University of Washington

We are interested in elucidating the precise role of IL-25 in the development of a TH2 response. Specifically, we would like to determine which cells are the main producers of IL-25, the cells it is capable of acting upon, and what effect it has on those cells. At mucosal sites, epithelial cells respond to environmental contaminants as well as infections by producing inflammatory cytokines IL-25, IL-33, and thymic stromal lymphopoietin (TSLP). IL-25 (IL-17E) is well known for its ability to induce TH2 cytokines (IL-4, IL-5, and IL-13) thereby enhancing the TH2 response, while correspondingly suppressing TH1 and TH17 responses. This leads to increased IgE production and eosinophilia, demonstrating its importance in both host defense to helminth infections as well as pathogenesis in allergic disorders. Cells capable of producing IL-25 include CD4⁺ and CD8⁺ T cells, mast cells, eosinophils, epithelial and endothelial cells. The IL-25 receptor is heterodimeric, consisting of IL17RA and IL17RB, and is expressed on T cells, macrophages, epithelial cells, and type 2 innate lymphocytes (ILC2s). Our lab has developed two novel mouse model systems to further investigate the biology of IL-25. The first was generated using a cDNA encoding IL-25 that was cloned into a Tetracycline Response Element (TRE) vector. The TRE-IL25 mouse was then crossed to a mouse containing a reverse tetracycline trans activator (rtTA) under the control of a clara cell 10 kD (CC10) promoter, giving us a lung specific IL-25 transgenic (referred to as CC10/IL25) upon treatment with doxycycline. The second is a conditional knockout in which the IL-25 receptor subunit specific to IL-25, IL-17RB, is flanked with LoxP sites. Crossing the IL-17RBfloxed mouse to a Cre recombinase expressing mouse allows us to abolish IL-25 signaling within specific cell types. These mice will play a central role in our ability to more specifically determine the cell types responding to IL-25 and generating a TH2 response. In CC10/IL25 transgenic mice, we found dramatic increases in lung IL-25 mRNA transcription levels by real-time PCR, IL-25 protein in lung and bronchial alveolar lavage (BAL) fluid, eosinophilia, and increased mucus production. Protein and gene expression levels for IL-33 and TSLP remained unchanged in transgenic mice, demonstrating an independent role for IL-25. Preliminary studies using wild-type mice indicate this response can occur independently of antigen; however, it is enhanced in the presence of antigen suggesting an effect on both innate and adaptive immunity. The novel CC10/IL25 transgenic and IL-17RBfloxed mouse models provide us with a unique opportunity to investigate the specific cellular subsets involved in promoting TH2 responses via IL-25.

Presenter: Thiemann, Sandra

Galectin-1 regulates dendritic cell tissue egress across lymphatic endothelial cells and migration of dendritic cells from inflamed tissue to lymph nodes

Sandra Thiemann, Jeanette H Man, Linda G Baum

University of California, Los Angeles

During inflammation, several dendritic cell (DC) populations migrate from inflamed tissue across lymphatic endothelium into lymphatic vasculature. DCs then traffic to regional lymph nodes where they initiate and regulate adaptive immune responses. However, the processes that regulate DC tissue egress and migration across lymphatic endothelium are ill defined. Our goal is to understand the role of glycan-lectin interactions in tissue egress of immunogenic and tolerogenic DCs (iDCs and tDCs). Galectin-1 (gal-1) is highly expressed in the extracellular matrix (ECM) and by endothelial cells in inflamed human and mouse tissue. In vitro, we have found that gal-1 in ECM selectively inhibits migration of human iDCs through ECM and across lymphatic endothelial cells (LECs), while human tDCs are not affected. We identified the major gal-1 counterreceptor on human DCs as the cell surface mucin CD43, and showed that differences in CD43 O-glycans on iDCs and tDCs regulate binding of gal-1 and are thus responsible for the difference in migration. The intracellular mechanisms by which gal-1 binding to CD43 retards iDC migration are currently being investigated. In order to understand the role of gal-1 in DC egress into the lymphatic vasculature under lymph stasis and inflammatory conditions in vivo, we used a lymphedema model. We compared lymphedema progression and lymph node DC numbers in a surgical lymphedema model of the tail using gal-1^{-/-} and wild-type control mice. Lymphedema developed faster and tissue damage was more severe in gal-1^{-/-} animals than in control animals. The total number of immune cells (DCs, B and T cells) in the draining lymph nodes was significantly increased in gal-1^{-/-} lymphedema animals compared to controls. We are currently characterizing the phenotype and function of the DCs that have migrated to the lymph node.

Presenter: Thompson, Lucas

**Bystander Activation of Na \tilde{A} ⁻ve CD4 T cells Via Type I Interferon Enhances
Differentiation of Foxp3+ Regulatory T Cells**

Lucas Thompson, Jen-Feng Lai, Andrea Valladao, Tenille Thelen, Zoe Urry, Steven F. Ziegler
Benaroya Research Institute

Not to be placed on website.

Presenter: Tinoco, Roberto

P-selectin glycoprotein ligand-1 limits the magnitude of T cell responses during chronic viral infection

Roberto Tinoco, Florent Carrette, Jonathan Magana, Susan Swain, Linda Bradley
Sanford-Burnham Medical Research Institute

Not to be placed on website.

Presenter: Tomala, Jakub

Design and production of chimeric protein of IL-2 linked to light chain of anti-IL-2 mAb S4B6 that structurally mimics IL-2/S4B6 immunocomplexes

Jakub Tomala, Jakub Tomala, Barbora Dvorakova, Jirina Kovarova, Martina Kabesova, Petra Votavova, Helena Chmelova, Blanka Rihova and Marek Kovar

Institute of Microbiology AS CR, Prague, Czech Republic

We designed and produced protein chimera consisting of IL-2 linked to light chain of anti-IL-2 mAb S4B6 through flexible oligopeptide spacer (Gly₄Ser)₃. It resembles IL-2/anti-IL-2 mAb immunocomplexes, which were reported to have significantly higher biological activity than free IL-2 *in vivo*. Depending on the mAb used they show different selectivity of their stimulatory activity. However, there are several disadvantages like possible dissociation of IL-2 from mAb or excess of either substance during preparation of these immunocomplexes. The intramolecular interaction of IL-2 and mAb part in our protein chimera is similar to that in immunocomplexes of IL-2 and anti-IL-2 mAb S4B6 (henceforth IL-2/S4B6), but dissociation of IL-2 too far away from mAb is prevented by its covalent attachment to S4B6 mAb. Such approach allows to exploit superior biological activity of IL-2 immunocomplexes and solve the postulated problems at the same time. We showed the produced protein chimera has predicted molecular weight slightly higher than IL-2/S4B6, contains both IL-2 and S4B6 mAb in one molecule and IL-2 interacts with binding site of S4B6 mAb *in cis*. Thus, we conclude that IL-2/S4B6 protein chimera mimics IL-2/S4B6 immunocomplexes in terms of structure and cytokine-mAb interactions. This work was supported by Czech Science Foundation grant P301/11/0325, 13-12885S and by Institutional Research Concept RVO 61388971.

Presenter: Traylor-Knowles, Nikki

Some Like It Hot: The Ancient Role of Tumor Necrosis Factor Receptor in Cnidarians

Nikki Traylor-Knowles, Nikki Traylor-Knowles, Stephen R. Palumbi

Stanford University

The tumor necrosis factor receptor (TNFR) is part of a super family of receptors responsible for the activation of a wide range of cellular pathways that are involved in stress response. Most notably, TNFRs can activate apoptotic pathways or immunity pathways, depending on the type of TNFR that is recruited. Current evidence suggests that the tumor necrosis factor receptor could have a very ancient role as an important regulator of the stress response in cnidarians (this phylum includes corals, and sea anemones). We hypothesize that the activation of the apoptosis initiating receptor is fast acting and short lived, while the immunity initiating receptor is activated more slowly, and has a longer time of expression. We intend to use several different cnidarian genomes, including *Nematostella*, to test this hypothesis and characterize the TNFR types that are found.

Presenter: Tyznik, Aaron J.

Simple Steps for Optimizing Multicolor Panel Design

Aaron J. Tyznik, Alan M. Stall, Maria C. Jaimes, Christian Carson, Jurg Rohrer

BD Biosciences

The use of flow cytometry has enabled the in-depth interrogation of complex cellular processes on a per cell basis. As the number of lasers available on cytometers and their accompanying reagents has increased, so has the potential for multiplexing and deeper analysis of cell populations and processes. However, choosing appropriate reagents to maximize the resolution of your data and performing multi-color flow, whether it is 6 fluorescent parameters or 16, requires consideration in selection of reagents. Here, we describe the process of performing a 6 color multi-color fluorescent experiment using 2 different approaches in panel design to help explain how your data is affected by the choices you make. Understanding the best practices in multi-color panel design enables better resolution of your data and can minimize experiment failure due to flow cytometric panel design flaws. Applying the simple panel design rules described here will help you to perform robust in-depth cellular analysis as represented by our 16 color cell surface and 10 color transcription factor analyses.

Presenter: Vatakis, Dimitrios

Cocaine modulation of quiescent T cells enhances HIV infection

Dimitrios Vatakis, Dhaval Dixit, Seyeon Cindy Youn, Irene Kim, Andrew Cross, Jeffrey Shen
David Geffen School of Medicine at UCLA, Department of Medicine, Division of Hematology-Oncology, UCLA AIDS Institute

Stimulant use such as cocaine has been shown to impact the human immune system. In regards to the human immunodeficiency virus (HIV) infection, a number of studies have indicated that cocaine users are at an increased risk for infection and display more rapid disease progression and morbidity. However due to many variables such as adherence to antiretroviral therapy, use of multiple classes of drugs and co-infections among others, it is difficult to fully appreciate the impact drug abuse has on HIV disease. We hypothesize that cocaine will influence the kinetics of HIV infection in quiescent cells by increasing their permissiveness to infection. To this end, quiescent cells were exposed to cocaine for three days. Based on our data, 3-day exposure, when compared to quiescent cells, resulted in increased reverse transcription kinetics, higher levels of viral cDNA, increased viral RNA and protein synthesis. In addition, the 3-day treated cells progressed to the G1b phase of the cell cycle and displayed a marked increase in the levels of CCR5. The cocaine effects were mediated via the Dopamine D4 and SIGMA-1 receptor pathways. The patterns of enhanced HIV infection were also observed in vivo using BLT humanized mice. Acute cocaine exposure of mice resulted in increased inflammation, accelerated kinetics of infection and higher viral loads. Thus, cocaine has a potentiating effect of HIV replication through increased permissiveness of resting T cells and increased immune activation.

Presenter: Waterfield, Michael

The transcriptional regulator Aire co-opts the repressive ATF7ip-MBD1 complex for induction of immune tolerance

Michael Waterfield, Imran S. Khan, Jessica T. Cortez, Joshua L. Pollack, David J. Erle, and Mark S. Anderson

University of California San Francisco

The maintenance of immune tolerance requires the deletion of self-reactive T cells during their development in the thymus. The expression of tissue-specific antigen genes (TSAs) by thymic epithelial cells is critical for this process and depends on the activity of the Autoimmune Regulator (Aire) protein. While Aire is known to be essential for TSA gene induction, the molecular mechanism(s) it uses to target TSA gene loci have remained obscure. Here we used an unbiased yeast two-hybrid approach to identify two novel Aire-interacting proteins involved in epigenetic regulation: activating transcription factor 7 interacting protein (ATF7ip) and methyl CpG binding protein 1 (MBD1). Chromatin immunoprecipitation (ChIP) and global gene expression profiling revealed that Aire co-opts the normally repressive ATF7ip-MBD1 protein complex to specifically target repressed TSA loci for induction. Furthermore, analysis of MBD1-deficient mice uncovered that MBD1 is required for the maintenance of immune tolerance by enabling the expression of Aire-dependent TSA genes in the thymus. These findings underscore the critical importance of Aire's interaction with the ATF7ip-MBD1 protein complex for central tolerance and the prevention of autoimmunity.

Presenter: Wu, Ting-Ting

Inhibition of type I IFN signaling by ORF10 of gamma-herpesviruses through a cellular mRNA export pathway

Ting-Ting Wu, Danyang Gong, Yoon Hoon Kim, Yafang Xie, Ronika Sitapara Leang, Stefanie Jaeger, Harding Luan, Sara Shu, Emily Duong, Leming Tong, Jennifer Truong, Seungmin Hwang, Sumit Chanda, Nevan Krogan, Ren Sun

UCLA

Persistent infections of human gamma-herpesviruses, Epstein-Barr virus (EBV or HHV-4) and Kaposi's sarcoma-associated herpesvirus (KSHV or HHV-8), are associated with several malignancies. Through co-evolution with hosts, herpesviruses have acquired many strategies to evade the host immune surveillance, effectively allowing their life-long persistence. ORF10, conserved among gamma-herpesviruses, blocks the signaling induced by type I interferons (IFNs). We found that ORF10 reduces the protein level of endogenous IFNAR chain1 (IFNAR1). We hypothesize that this activity of the viral genes leads to the inhibition of type I IFN signaling. To elucidate the molecular mechanism, we sought to identify the interacting proteins of ORF10 by using co-immunoprecipitation followed by mass spectrometry. The interaction with the cellular mRNA export protein, Rae1, was identified and further validated. Nuclear accumulation of poly(A) RNA was observed when cells were infected with a murine gamma-herpesvirus expressing ORF10 or in cells expressing ORF10 alone. This alteration of poly(A) distribution was reversed by mutants of ORF10 protein that fail to interact with Rae1 or partially reversed in cells infected with a mutant virus lacking ORF10. Interestingly, ORF10 decreases the cytoplasmic/nuclear RNA ratio of IFNAR1 but not that of IFNAR2. This is consistent with the data that protein down-regulation was only observed for IFNAR1 but not IFNAR2. Together, we propose that one of the mechanisms by which gamma-herpesviruses modulate the host immune responses is to specifically down-regulate cellular gene expression by selectively interfering with mRNA export.

Presenter: Xu, Yuexin

Optogenetically Engineered T Cells for Cancer Immunotherapy

Yuexin Xu, Yuexin Xu, Young-Min Hyun, Kihong Lim, Hyunwook Lee, Ryan J. Cummings, Scott A. Gerber, Seyeon Bae, Yoonsang Cho, Edith M. Lord, and Minsoo Kim

University of Rochester Medical Center

Chemokine receptors are promising therapeutic targets because chemokine mediated cell migrations play key roles in mediating leukocyte differentiation, survival, and tissue-specific homing. However, although chemokine receptors are predominantly expressed on leukocytes, they are also widely expressed on many other cell types. For example, in tumor environment, effective trafficking of the cytotoxic T lymphocytes (CTLs) to tumor sites is one of the main barriers to achieving successful melanoma remission. However, local induction of chemokine signaling to bring more CTLs to tumor site will also induce tumor cell survival and proliferation, angiogenesis and promoting tumor metastasis. To address this issue, we developed a strategy for optically controlling chemokine-mediated T cell trafficking in vivo. The intracellular loops of G_i-coupled rhodopsin were replaced with those of the G_{αi}-coupled chemokine receptor CXCR4. Photoactivatable CXCR4 (PA-CXCR4) transmitted intracellular CXCR4 signals in response to 505-nm light. Localized activation of PA-CXCR4 induced T cell polarization and directional migration (“phototaxis”) both in vitro and in vivo. When combined with adoptive transfer therapy, photoactivatable chemokine receptors could recruit CTLs to the location of a tumor following non-invasive light stimulation. Therefore, the photoactivatable chemokine receptors will allow us to precisely control spatiotemporal leukocyte trafficking during immune reactions in live animals.

Presenter: Yamada, Douglas

Impaired Antibody Effector Function During Persistent Viral Infection

Douglas Yamada, Heidi Elsaesser, David G. Brooks

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Not to be placed on website.

Presenter: Zaitseva, Galina

The effect of chronic exposure to sublethal doses of endosulfan on the expression of IL-6, IL -10 and TNF-a and histopathological changes in a mouse model of colon cancer.

Galina Zaitseva, Zaitseva GP, Chavez-Rubio LA, Peregrina-Sandoval J, Tellez- Banuelos MC

Universidad de Guadalajara

Introduction. Endosulfan, a pesticide widely used in Mexico, has been shown to have immunotoxic effects and may also facilitate the development of cancer. Objective. To assess the effect of chronic exposure to sublethal doses of endosulfan on the expression of cytokines IL-6, IL 10 and TNF-a and histopathological changes in mouse model of colon cancer. MATERIALS AND METHODS. Commercially-sourced Endosulfan was diluted in olive oil and administered (2mg/kg) to mice of both sexes via oral gavage for 20 weeks. In the control group only olive oil was administered. Colon cancer was induced in two groups (with and without endosulfan) using 1,2-dimethylhydrazine (DMH) 20mg/kg. Over a ten month period, two mice/month from each experimental group were euthanized with 0.1ml subcutaneous pentobarbital. Serum levels of the cytokines IL-6, IL-10 and TNF-alpha were determined using a commercial ELISA kit (Abcam). The colons were fixed in 10% formalin and processed for histology using the protocol of Propath et al, (1992); the resulting sections were stained using a conventional hematoxylin-eosin protocol (Moreno et al, 2007). The immunohistochemical staining was performed using monoclonal antibodies directed to P-selectin and β -catenin and developed with an avidin-biotin kit. RESULTS. The LD50 of endosulfan was determined to be 8mg/kg for females and 11.5 mg/kg for males. The sublethal dose used in this study caused acute inflammation of the colon and significant increases of serum IL-6 and P-selectin expression in the colon from first month of pesticide administration in females and from the second month of administration in males. The inflammation of the colon evolved over time to severe chronic colitis with high levels of TNF-a and precancerous elements after 3-4 months post endosulfan exposure. Serum IL-10 was found to be increased by 4 months post-exposure. At six months post-exposure, β -catenin was found to be strikingly expressed in aberrant crypts. At seven to eight months post-exposure, cancer in situ appeared in mice given both endosulfan and DMH. In contrast, in the DMH alone control adenocarcinoma in situ was detected only at 10 months post-exposure. CONCLUSIONS. Chronic exposure to endosulfan, even at very low doses, induces over-expression of molecules that indicate that an expanded inflammatory process in the colon causes severe chronic colitis, and over time, cancer. Additionally, administration of Endosulfan accelerates development of adenocarcinoma in a DMH model of colon cancer. These effects were observed in both sexes, but was more rapid and more pronounced in females.

Presenter: Zhang, Zhifang

CEACAM1 regulation of IL-6 after LPS challenge

Zhifang Zhang, Frances Chang, Keith Le, Courtni Salinas, Angel Gu, Jennifer Chean, John E. Shively

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Previously, we showed that CEACAM1 regulates IL-1 β production in LPS treated neutrophils in vitro in a TLR4-Syk specific manner (1). When comparing the level of circulating cytokines in wild type vs CEACAM1 KO mice treated with 10 mg/kg LPS (i.p.), IL-1 β , TNF α , MCP-1 and IFN γ show similar kinetics, while IL-6 levels are significantly elevated in CEACAM1 KO mice over 24h, returning to baseline by 48h. Phenotypically, after challenge with LPS, 53% CEACAM1 KO mice (9 out of 17) develop diarrhea in comparison with none in wild type mice. Both the wild type and CEACAM1 KO mice have depressed surface body temperature, with both related phenotypes exhibiting a prolonged course in the CEACAM1 KO mice. Recently, the source of IL-6 production in LPS treated mice has become controversial. Indeed, production of IL-6 was not statistically different between wild type and CEACAM1 KO mice in most organs, including liver, spleen, intestine, omentum, pancreas, and adipose tissue of the peritoneal cavity. Bone marrow monocytes, but not neutrophils produced significant amounts of IL-6 for 5 hrs after in vitro LPS plus Brefeldin A treatment. In vivo at 5 hr post LPS injection plus Brefeldin A, neither group produced IL-6, suggesting that these cells had exited bone marrow. In agreement, spleen monocytes from CEACAM1 KO mice had a higher percentage of IL-6 than wild type, and the percentage was even higher for neutrophils. We are studying the possibility that the in vivo production of IL-6 from neutrophils plays a major role in LPS treated mice, mechanistically connecting CEACAM1 to IL-6 production in these cells. REFERENCES 1. Lu, R., Pan, H., Shively, J. E. CEACAM1 negatively regulates IL-1 β production in LPS activated neutrophils by recruiting SHP-1 to a SYK-TLR4-CEACAM1 complex. PLoS Pathogen, 8(4): e1002597