Presenter: Arora, Pooja

Mechanism of cytokine bias in natural killer T cell responses to diverse glycolipid antigens

Pooja Arora, GS Besra, SA Porcelli

Albert Einstein College of Medicine

CD1d-restricted Natural Killer T cells (NKT cells) are a subset of T lymphocytes that recognize glycolipid antigens, and respond by secreting both the pro-inflammatory Th1- or anti-inflammatory Th2-cytokines. Several variants of prototypical glycolipid antigen, alpha-galactosylceramide (AGC), have been described that induce responses in which either the Th1 or Th2 cytokines predominate. It has been proposed that the variation in cytokine responses with different glycolipid antigens could reflect their presentation by different cell types. By using a monoclonal antibody that selectively recognizes CD1d/AGC complexes, we have shown that a single type of cell, the CD8APos DEC-205Pos dendritic cell, was mainly responsible for capturing and presenting a variety of different glycolipid antigens, including multiple forms of a-galactosylceramide that stimulate Th1 or Th2-biased cytokine responses. After glycolipid presentation, these dendritic cells rapidly altered their expression of various costimulatory and co-inhibitory molecules in a manner that was dependent on the structure of the antigen. These findings show flexibility in the outcome of two-way communication between CD8APos DCs and iNKT cells, providing a mechanism for biasing towards either pro-inflammatory or anti-inflammatory responses.

Presenter: Arroyo, Nicole

Antigen-specific CD4+ T cell differentiation in murine malaria infection

Nicole Arroyo, Marion Pepper

University of Washington

Malaria is a parasitic disease that kills approximately one million people a year. Although efforts to induce protection from malaria through vaccination have been ongoing for over sixty years, none have produced long-lasting immunity in malaria-endemic areas. This is likely due to a lack of knowledge of the immunological mechanisms required for protection. While it is clear that both CD4+ T cells and B cells are important contributors to immunity against the blood stage of infection, little is known about their specific functional contributions. We have used a murine model of Plasmodium infection, P. yoelii, to gain an understanding of the lymphocyte populations that form and are required for protection in both a primary and secondary infection. Using transgenic parasites and MHC class II tetramers to track CD4+ T cell responses during malaria, we have found that early in the response, the vast majority of CD4+ T cells that form are T follicular helper (Tfh)-like cells, despite the lack of a germinal center. We are currently using these tools to understand whether these cells are pathogenic or protective and how interactions with B cells drive their formation and expansion. Preliminary data further suggests that interactions between CD4+ T cells and B cells may be pathogenic during the early stage of the infection, but are required for parasite clearance. An effective vaccine may need to stimulate both the formation of antigen-specific T effector (Teff) and Tfh cells in order to provide effective immunity against rechallenge.

Presenter: Au-Yeung, Byron

Thresholds for Zap70 activity in T cell development and proliferation

Byron Au-Yeung, Heather Melichar, Julie Zikherman, Jenny Ross, James Mueller, Debra Cheng, Yiling Chen, Kevan Shokat, Ellen Robey, and Arthur Weiss

University of California, San Francisco

Upon TCR stimulation, it is generally accepted that Zap70 promotes the activation of downstream signal pathways associated with T cell activation. Less clear is the amount of Zap70-dependent signaling required to drive cellular responses. To quantitatively perturb Zap70 activity, we developed a chemical-genetic system in which a catalytically competent mutant of Zap70 can be selectively inhibited by a small molecule inhibitor. We also use a Nur77-GFP reporter transgene, which is expressed in relative proportion to TCR signal magnitude or duration. Stimulation of thymocytes or mature T cells in the presence of graded concentrations of Zap70 inhibitor resulted in similarly graded reductions in reporter expression. Using a model system of synchronized thymocyte positive selection, we showed that DP cells accumulate Zap70-dependent signals over 36 continuous hours to complete positive selection. Zap70 inhibitor titration resulted in graded decreases in positively selected cells. However, positively selected cells accumulated a relatively invariant "amount" of TCR signaling (Nur77-GFP expression), regardless of the concentration of Zap70 inhibitor. These results suggested a level of heterogeneity within a population of thymocytes, even if they all express the OT-I TCR. In mature CD4 and CD8 T cells, we also showed that titration of Zap70 inhibitor concentration quantitatively reduced the TCR signaling accumulated by activated T cells, and decreased the proportion of cells that enter the proliferative response. However, cells that divided also integrated an invariant amount of TCR signaling (Nur77-GFP) regardless of inhibitor concentration. Our studies indicate the existence of TCR signaling thresholds for development and activation, and that such thresholds remain invariant despite tuning of TCR signal magnitude and duration.

IL-6 and IL-27 exert unique and overlapping effects of CD4+ T cells during chronic viral infection

Burton Barnett, James Harker, Phuc Bao, Aleksandr Dolgoter, Elina Zuniga

University of California, San Diego

Persistent viruses such as human immunodeficiency virus and hepatitis C and B viruses are associated with diminished cytotoxic and antibody responses, which in spite of their delayed and weakened activity, exert a limited level of viral control. It is understood that CD4+ helper T cells are central to establishing effective cytotoxic CD8+ T cell and antibody mediated immunity. Therefore, understanding the molecular factors that control CD4+ T cells during chronic viral infection is of utmost importance. By using lymphocytic choriomeningitis virus (LCMV) clone 13 infection in mice as a model system, we set out to investigate the role of interleukin-6 (IL-6) and IL-27 in regulating CD4+ T cell responses during chronic infection. To overcome potential redundancies between these two cytokines we examined IL6R and IL27R double knockout mice infected with LCMV clone 13. We observed reduction in the number of virus-specific CD4+ T cells and compromised TFH responses and IL-21 production, which become evident by day 30 after infection. These CD4+ T cell deficiencies were cell-intrinsic, as shown by mixed bone marrow chimera experiments, and were accompanied by cell-extrinsic defects in CD8+ T cell responses, GC B cell responses, lower virus specific antibody titers, and impaired viral control. In IL-6R and IL-27R single knockout mice, TFH and CD4+ T cell survival (but not IL-21 production) were affected, respectively. Together, these data support a model in which IL-6 and IL-27 exert unique (i.e. TFH and survival) and redundant (IL-21 induction) effects in virus specific CD4+ T cells at late stages during chronic viral infection.

Presenter: Barry, Kevin C.

Translation inhibition by bacterial pathogens: Links to 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: Bergsbaken, Tessa

Proinflammatory microenvironments within the intestine regulate the differentiation of CD8 T cells responding to infection

Tessa Bergsbaken, Michael J. Bevan

University of Washington

CD8 T cells in mucosal tissues play an important role in controlling infection by a variety of pathogens. We report that oral infection with the enteric pathogen Yersinia pseudotuberculosis (Yptb) results in two distinct populations of pathogen-specific CD8 TRM cells in the lamina propria (LP). Surprisingly, one population did not require TGFÃŸ signaling and did not express CD103. Despite these unusual features, CD103neg T cells were true resident memory cells, as they were long lived and resistant to systemic depletion. Unlike the CD103+ CD8 T cells, which were TGFÃŸ-dependent and scattered in the tissue, CD103neg T cells were clustered with CD4 T cells and CX3CR1+ macrophages/dendritic cells around areas of bacterial infection. CXCR3-dependent recruitment to areas of inflammation was critical for development of the CD103neg population and for pathogen clearance. These studies have identified the preferential development of CD103neg LP TRM cells in inflammatory microenvironments within the LP and suggest that this subset plays a critical role in controlling infection.

The role of neutrophils and extracellular adenosine in shaping host resistance to Streptococcus pneumoniae lung infection

Elsa Bou Ghanem, Stacie Clark, Sara E. Roggensack, Sally R. McIver, Pilar Alcaide, Phil G. Haydon and John M. Leong

Tufts University

Streptococcus pneumoniae (pneumococcus) invasive infections, such as pneumonia, bacteremia and meningitis, remain a leading cause of mortality and morbidity worldwide despite the availability of a vaccine. An important determinant of the course of disease following S. pneumoniae infection is polymorphonuclear leukocyte-(PMN-) mediated pulmonary inflammation. However, different studies report that, during pneumococcal infection, these innate immune cells may promote either immune protection or severe disease. By careful monitoring of the influx of PMNs throughout the course of infection, we found that upon intratracheal challenge of mice, early PMN influx coincided with a decrease in pneumococcal burdens in the lungs, but continued inflammation correlated with increased pulmonary and blood-borne pneumococci. Depletion of PMNs prior to lung challenge significantly increased the susceptibility of mice to S. pneumoniae, but PMN depletion at peak infiltration 18 hours post-infection lowered bacterial numbers a 100-fold in both the lungs and blood and enhanced survival. Thus, our findings demonstrate that optimal host defense against pneumococcal disease requires an initial robust PMN response followed by modulation of this response later in infection. To better understand the signals that regulate the influx of PMNs into the lungs during pneumococcus infection, we investigated a crucial regulator of pulmonary inflammation, extracellular adenosine (EAD), whose production requires the cell surface enzyme, 5'-nucleotidase CD73. We found that diminishing EAD production by pharmacological inhibition or genetic ablation of CD73, or by chemically blocking EAD signaling receptors led to dramatically increased accumulation of PMNs in the lungs upon S. pneumoniae infection of mice. Importantly, blocking EAD production or signaling also increased the susceptibility of mice to systemic pneumococcal infection. This susceptibility of CD73-deficient mice was reversed by PMN depletion following infection, suggesting that control of PMN influx is a central factor in EAD-mediated resistance. Our findings identify EAD as a negative regulator of pulmonary PMN influx, an activity that is crucial for innate host resistance to S. pneumoniae.

Presenter: Bruchez, Anna

Transposon Mutagenesis Screen to Identify Host-Encoded Anti-Viral Factors

Anna Bruchez, Ky Sha, Li Chen, Adam Lacy-Hulbert, Lynda Stuart

Benaroya Research Institute, Massachusetts General Hospital

Ebola and Lassa virus cause sporadic outbreaks of severe hemorrhagic fever. Owing to their high mortality rates, they pose significant health risks and are potential bioterrorism agents. However, our knowledge of how they usurp host cells to complete their life cycle, and more importantly, how this can be combated, is limited. Here we describe a new genome-wide approach to identify genes and pathways that confer resistance to infection. This work will provide insights into host anti-viral defense mechanisms and may be used to identify new therapeutic targets that can be manipulated to promote host-encoded anti-viral immunity. We are using a forward genetics approach that our lab previously developed to identify host genes, known as restriction factors, which confer resistance to viral infection. In this system cells are mutagenized with a transposon that activates genes close to its insertion site. Libraries of mutagenized cells are screened with a cytotoxic virus and resistant cell populations are selected. Illumina sequencing is then used to characterize transposon insertion sites and identify candidate resistance genes. Using this method we have performed a screen in the human cell lines A549, U-2 OS, HEC-1-B, HELA and 293T for genes conferring resistance to either vesicular stomatitis virus (VSV) or recombinant VSV bearing either the Ebola virus (Ebo-VSV) or Lassa fever virus surface glycoprotein (LFV-VSV). We have identified genes associated with resistance to only one type of virus, or to multiple types, demonstrating that resistance can occur through targeting of either viral entry or viral replication. One of the genes our screen identified is NPC1, a known cofactor for Ebola virus entry; thus, confirming that this is a valid method of identifying virus-host interactions. We are currently characterizing the novel candidates to confirm resistance genes, and extending the screen to additional cell lines and viruses. Supported by NIAID Grant R21AI102266.

Non-typhoidal Salmonella Typhimurium ST313 isolates that cause bacteremia in humans stimulate less inflammasome activation than ST19 isolates associated with gastroenteritis.

Sarah Carden, Chinyere Okoro, Gordon Dougan, Denise Monack

Stanford University School of Medicine, Wellcome Trust Sanger Institute

Salmonella is an enteric pathogen that causes a range of diseases in humans. Non-typhoidal Salmonella (NTS) serovars such as Salmonella enterica serovar Typhimurium generally cause a self-limiting gastroenteritis whereas typhoidal serovars cause a systemic disease, typhoid fever. However, S. Typhimurium isolates within the multi-locus sequence type ST313 that commonly cause bacteremia in humans have emerged in sub-Saharan Africa. S. Typhimurium ST313 are phylogenetically distinct from classical S. Typhimurium lineages such as ST19 that cause zoonotic gastroenteritis worldwide. Previous studies have shown that the ST313 lineage has undergone genome degradation when compared to the ST19 lineage, similar to that observed for typhoidal serovars. Currently, little is known about phenotypic differences between ST313 isolates and other NTS isolates. We find that ST313 isolates invade non-phagocytic cells less efficiently than the classical ST19 isolates that cause gastroenteritis. In addition, ST313 isolates induce less Caspase 1- dependent macrophage death and IL-1ÃŸ release than ST19 isolates. ST313 isolates also express lower levels of mRNA of the genes encoding the SPI-1 effector sopE2 and the flagellin, fliC, providing possible explanations for the decrease in invasion and inflammasome activation. The ST313 isolates have invasion and inflammatory phenotypes that are intermediate; more invasive and inflammatory than Salmonella enterica serovar Typhi and less than ST19 isolates associated with gastroenteritis. This suggests that both phenotypically and at the genomic level ST313 isolates are evolving to become more similar to typhoidal serovars and adapting to cause systemic disease in humans.

Presenter: Casbon, Amy-Jo

Tumors Reprogram Early Myeloid Differentiation in the Bone Marrow to Generate Immunosuppressive Neutrophils

Amy-Jo Casbon, Amy-Jo Casbon, Damien Reynaud, Chanhyuk Park, Emily Khuc, Dennis D. Gan, Koen Schepers, Emmanuelle PasseguÃ© and Zena Werb.

University of California, San Francisco

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A whole genome mouse siRNA screen to identify novel genes involved in lipid antigen presentation

Shilpi Chandra, Archana Khurana, James Gray, Sonia Sharma, Bjoern Peters, Mitchell Kronenberg

La Jolla Institute for Allergy and Immunology, San Diego

Although most T lymphocytes recognize peptides presented by major histocompatibility complex (MHC)-encoded class I and class II molecules, there also are significant populations of T cells that recognize nonpeptide antigens. Prominent among these T lymphocytes are the type I or invariant natural killer T cells (iNKT cells). These T lymphocytes recognize lipids presented by CD1d, a nonpolymorphic, class I-like, antigen-presenting molecule. We have carried out a whole genome siRNA screen in a macrophage cell line for genes that affect the presentation of a potent glycosphingolipid antigen, GalGal Cer, to iNKT cells. In order to stimulate iNKT cells, this antigen requires internalization and lysosomal carbohydrate antigen processing to remove the terminal galactose. After several rounds of validation, functional classification and gene expression analysis, we have identified genes that lead to altered antigen presentation in macrophages. A majority of the identified genes do not perturb surface CD1d expression, but we can demonstrate they effect the formation of surface CD1d complexes with the stimulating glycolipid, and their absence does not have any effect on MHC class I or class II antigen presentation pathways. Therefore our data indicate that the CD1d and MHC class II antigen presentation pathways are highly divergent, although both depend on antigen loading in endolysosomal compartments.

Presenter: Chandra, Vivek

In lymphoid progenitors, a novel lncRNA, termed AJAX, repositions the EBF1 locus from the heterochromatic to the euchromatic compartment, to orchestrate early B cell development

Vivek Chandra, Marty Flores and Cornelis Murre

University of California, San Diego, USA

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Presenter: Chen, Runqiang

A pooled RNA interference screen in vivo identifies regulators of antiviral CD8+ T cell differentiation

Runqiang Chen, Simon Belanger, Megan A. Frederick, Bin Li, Sonia Sharma, Bjoern Peters, Anjana Rao, Shane Crotty, Matthew E. Pipkin

La Jolla Institute

Memory CD8+ T cells play an essential role in long-term immunity against intracellular infections, but our understanding of the cell-intrinsic molecules that control their differentiation is limited. To identify previously unrecognized transcriptional regulators that control effector and memory CD8 T cell development in vivo, we developed a pooled approach to screen 30-100+ genes individually in antigen-specific T cells during infection using short hairpin RNAs in a microRNA context (shRNAmir). In vivo screen using T cell receptor (TCR)-transgenic CD8+ T cells responding to lymphocytic choriomeningitis virus (LCMV) identified both known and new genes that regulated short-lived effector and memory precursor cytotoxic T lymphocytes (CTLs). The screen revealed the role for the positive transcription elongation factor (P-TEFb) component Cyclin T1 (Ccnt1) in effector and memory CTLs differentiation in vivo. Inhibiting expression of Cyclin T1, or its catalytic partner Cdk9, impaired development of protective short-lived effector CTL and enhanced memory precursor CTL formation in vivo. This pooled shRNA screening approach should accelerate the understanding of T cell differentiation in vivo.

Presenter: Cho, Sunglim

miR-23~27~24 clusters restrict Th2 immunity and associated immunopathology during airway allergic reaction

Sunglim Cho, Chen-Jang Wu, Tomoharu Yasuda, Leilani Cruz, Ling-Li Lin, Marina Miller, Hyang-Mi Lee, David Broide, Klaus Rajewsky, Li-Fan Lu

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MiRNAs (miRNAs) are important regulators in T cell differentiation and function. Here we show miR-23~27~24 clusters play a pivotal role in controlling type II immunity. Specifically, under Th2 polarizing condition, T cells with overexpression of miR-23~27~24 clusters exhibited reduced IL-4 secretion whereas T cells devoid of miR-23~27~24 clusters produced elevated amounts of IL-4. Further mechanistic studies revealed miR-24 and miR-27 could repress Gata3, a key regulator of Th2 cell differentiation through an indirect and a direct manner, respectively. Finally, by using an OVA-induced asthma model, we have shown that mice with T cell-specific ablation of miR-23~27~24 clusters developed a more severe airway inflammation characterized by increased IL-4 secretion, lung eosinophil infiltration, mucin production and serum IgE levels. Taken together, our studies identify a miRNA family with important biological function particularly in controlling Th2 immunity and suggest that a tight regulation of this miRNA family is required to maintain optimal effector T cell function and to prevent aberrant immune responses.

Parasite-induced Alternative Activation of Host Cells Regulates Immune Pathology During Infection

David Christian, Lucas A. Dawson, Anita A. Koshy, Daniel P. Beiting, Jung Oh, David R. Roos, David Artis, Christopher A. Hunter

University of Pennsylvania

In response to T. gondii infection, the host develops a robust Th1 response to produce IFN-g, which is required for control of this intracellular parasite. It is well established that during infections with Type II strains of T. gondii, the regulatory cytokines IL-10 and IL-27 are required to prevent lethal immune pathology mediated by CD4+ T cells. However, during infection with Type III strains, mice that lack IL-10 or IL-27 develop similar degrees of weight loss as Type II infections but survive this challenge. These data indicate the presence of a mechanism that is independent of IL-10 and IL-27 that limits immune pathology that is specific to Type III infections. One of the critical differences between these two strains is that the Type IIII strains express a kinase (rhoptry protein 16, ROP16) that is injected into host cells and which results in prolonged activation of the host transcription factor STAT6. These events lead to the induction of an alternatively-activated phenotype of macrophages and DCs that is STAT6-dependent but independent of IL-4 or IL-13 signals. Using transgenic parasites and reporter mice to identify injected host cell populations, these studies revealed that challenge with type III strains was characterized by the presence of large numbers of uninfected DCs and macrophages that were injected and expressed an alternatively-activated phenotype characterized by the expression of RELMa and CD206. Studies on the role of these injected cells to control immune pathology during infection with Type III strains of T. gondii will be discussed.

Presenter: Clingan, Jonathan

RNase L is a specific regulator of protein translation and limits activation of the RLR-mediated antiviral response

Jonathan Clingan, Sterling C. Eckard, Daphne A. Cooper, Hilario J. Ramos, Jaime Guillen-Casas, Bruno Canard, Michael Gale, Jr., Jay R. Hesselberth, David J. Barton, and Daniel B. Stetson

University of Washington

Intracellular detection of foreign DNA or RNA in mammalian cells triggers distinct sensors that protect against virus infection: DNA activates the cyclic GMP-AMP synthase (cGAS)-STING pathway, whereas RNA activates the RIG-I-like receptor (RLR)-MAVS pathway. In addition, a family of enzymes called oligoadenylate synthetases (OAS) detect foreign RNA and catalyze the formation of 2'-5'-linked oligoadenylates (2-5A). The sole known biological function of 2-5A is to activate the endoribonuclease RNase L, which is thought to cleave cellular and viral RNAs indiscriminately into small immunostimulatory fragments that amplify the RLR response. Here, we show that contrary to this prevailing view, RNase L-deficient cells have an enhanced antiviral response to RLR ligands. We find that the in vivo specificity of RNase L is restricted to several unique sites in ribosomal RNA and that RNase L activation leads to rapid depletion of translating ribosomes, thus establishing RNase L as a specific regulator of protein translation, not an indiscriminate mediator of RNA decay. Moreover, we demonstrate that 2-5A molecules are endogenous activators of the innate antiviral response. These findings reveal that 2-5A made by OAS enzymes has differential roles for regulating the innate immune response via both RNase L-mediated translational suppression, and a direct immunostimulatory role for 2-5A itself.

Presenter: Cohn, Benjamin

Effect of TGF-[b] on the CD8 T cell response to rectal LCMV infection

Benjamin Cohn (1,2), Timothy Borbet (3), Julie Luong (1), Erik Woodruff (1), Shahzada Khan (1), Martin Trapecar (1), Shomyseh Sanjabi (1,4)

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Transforming growth factor (TGF)-B is a pleiotropic cytokine involved in both suppressive and inflammatory immune responses. In the colon, TGF-B plays a pivotal role in maintaining tolerance against innocuous food and commensal bacteria, but little is known about how TGF-B affects the anti-viral immune response against rectally transmitted pathogens. We have previously shown that TGF-B signaling selectively causes apoptosis in CD127-KLRG1+ short-lived effector cells, leading us to predict that this effect may be amplified in the TGF-B-rich environment of the gut. To address the role of TGF-B in colon immunity, we have established a model for rectal lymphocytic choriomeningitis virus (LCMV) infection in mice, allowing us to compare the rectal immune response to the systemic response elicited against LCMV. We demonstrate that LCMV is transmitted rectally without causing any intestinal damage, and passes from the colon to the iliac (iLN) and mesenteric draining lymph nodes. Compared to the systemic route, CD8 T cell priming and activation is delayed in rectal infection: mucosal priming occurs in the iLN 3 days post rectal inoculation, followed by a systemic activation 5 days post infection. The kinetics of CD8 T cell activation correlate with the kinetics of viral transmission and the amount of time it takes the virus to spread from the rectum to the draining lymph nodes. Using mice carrying a CD8 T Cell-specific deletion of TGF-B Receptor II (RII), we determined the effect of TGF-B signaling on three distinct phases of the CTL response: priming within regional lymph nodes, clonal expansion and memory formation. Determining the effect of TGF-B on the mucosal anti-viral CD8 T cell response will help us understand the requirements for viral control in the colon and other mucosal tissues. A better understanding of how immunological tolerance is overcome in mucosal tissues such as the colon will pave the way for effective therapies against HIV and other sexually transmitted viral infections.

Evolutionary Tradeoffs Between RNA Interference and Rig-I Like Receptors

John Crowl, Hannah Volkman, Dan Stetson

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 have found that one of them activates a potent type I Interferon response dependent on RLR signaling and the RdRPâ€™s catalytic activity.

Presenter: Cruz, Leilani

Role of miR-27 in controlling T cell immunity

Leilani Cruz, Sunglim Cho, Chen-Jang Wu, Tomoharu Yasuda, Ling-Li Lin, Klaus Rajewsky, Li-Fan Lu

University of California, San Diego

miR-23~27~24 clusters are highly enriched in regulatory cells while expressed at low levels in conventional T cells. However, despite a selective expression pattern of miR-23~27~24 clusters in T cells, to date, studies of this miRNA family have primarily focused on their role in tumorigenesis. Here, we show miR-23~27~24 clusters play a pivotal role in regulating T cell immunity. Particularly, our results have demonstrated that the majority of miR-23~27~24 cluster-dependent phenotypes could be attributed to a single member of this miRNA family, miR-27. Mice with T cell-specific overexpression of miR-27 spontaneously developed autoimmune phenotypes. On the other hand, in vitro polarization studies revealed that T cells with miR-27 overexpression exhibited impaired differentiation and effector function of multiple T helper cell lineages. Finally, our mixed bone marrow chimeras studies further demonstrated that miR-27 could control different T cell responses through either a T cell-intrinsic or -extrinsic manner. Collectively, Our results identify a new

Immunoregulatory Dendritic Cells During Persistent Viral Infection

Cameron R. Cunningham, Elizabeth B. Wilson, David G. Brooks

University of California, Los Angeles

Immunosuppressive factors drive the immune dysfunctions that prevent virus clearance thereby enabling viral persistence. Ablation of these suppressive pathways restores immune activity facilitating enhanced control of the persistent virus. Understanding the sources, developmental cues, and mechanisms of induction of the suppressive factors will enable directed approaches to specifically block these pathways to augment an immune response crippled by suppression. Recently, we identified a specific immunoregulatory antigen presenting cell (iregAPC) population during persistent lymphocytic choriomeningitis virus (LCMV) infection in mice that specifically produces potent immunoregulatory factors, including IL-10, PD-L1, and indoleamine 2,3-dioxygenase (IDO), that suppress antiviral T cell responses. Interestingly, we recently demonstrated that in addition to its critical antiviral role throughout viral persistence, sustained type I interferon (IFN-I) signaling led to many of the suppressive mechanisms (including IL-10 and PD-L1 expression) and immune dysfunctions associated with persistent viral infections. Yet, how the iregAPC are generated, acquire suppressive activity (and if these events are separable), and the role of IFN-I in this process were all unclear. To interrogate iregAPC, we identified protein expression unique to these cells and used these to demonstrate that IFN-I suppresses the expansion of stimulatory DCs during persistent LCMV infection, while simultaneously endowing suppressive activity to iregAPC. However, direct IFN-I signaling was not required for the development of iregAPC, but instead generation of this population was dependent on MyD88 and IFNγ signaling. We are currently investigating the molecular mechanisms and cellular interactions through which MyD88/IFNγ drive the emergence of this population and why these cells are susceptible to IFN-I mediated induction of an immunosuppressive function while other APC are not. Thus, our data indicate that immunosuppression during viral persistence is a multi-step process wherein MyD88 and IFNγ signaling lead to the development of a population of APC with suppressive potential and that ongoing IFN-I signaling then secondarily endows this suppressive function. Ultimately, we anticipate that these studies and future exploration will provide a greater understanding of the sources and dynamics of regulatory factors produced during a persistent infection and potentially identify novel therapeutic targets to boost an otherwise suppressed immune response.

Presenter: Curtale, Graziella

Mechanisms of strand selection of microRNAs in T lymphocytes

Graziella Curtale, Matthew E. Pipkin

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MicroRNAs (miRs) are important post-transcriptional modulators of gene expression and cell differentiation. However, the mechanisms that determine which strand of the precursor miRs (pre-miRs) that ultimately get loaded into the RNA induced silencing complex (RISC) are still unclear. Here we show that whereas miR-125a-5p is predominantly loaded into the RISC in human cells, an "arm-switching" mechanism appears to exist such that the opposite miR-125a-3p strand is the predominant sequence loaded in the RISC in murine macrophages and CD8 T cells. Sequences of the human and mouse pre-miR-125a are identical, but the less mature pri-miR-125a forms differ at several bases in the 5'- and 3'-flanking regions of the pre-miR between species, suggesting mechanisms other than pre-miR thermodynamic instability accounts for specific strand loading. Notably, the flanking sequences of the murine pri-miR-125a are sufficient to switch loading of 5p and 3p arms of a heterologous miR-146b, suggesting that pre-miR flanking sequences influence strand loading. We are combining next generation sequencing and a two reporter sensor assay that detects loading of either 5p or 3p strands of a specific miR, to identify sequences that preferentially promote loading of the different miR arms from a library of pri-miRs encoding miR-146b with random flanking sequences in the context of CD8 T cells. These studies are likely to shed light on how miR strand selection is achieved and how regulation of target gene specificity is governed during immune cell development and function.

Presenter: De Leon, Justin

Identifying factors that inhibit host protein synthesis during Legionella pneumophila infection

Justin De Leon, Kevin C. Barry, Jonathan L. Portman, Mary F. Fontana, and Russell E. Vance

University of California, Berkeley

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Presenter: Deshpande, Neha

Affinity for self drives the preferential accumulation of promiscuous CD4+ T cells over the lifespan

Neha Deshpande (1), Sing Sing Way (2) and Michael S. Kuhns (1)

(1) The University of Arizona, (2) Cincinnati Children's Hospital Medical Center (2).

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Immunological method for measuring LDL-Cholesterol Using the Streptolysin O and Apolipoprotein B-100 anitibody

Kim Dowon

Hallym University

Cholesterol is one of the important components for necessary to maintain the body. It is related with function as component of cell membranes and it is also important raw material of sex hormone and cortical hormone. For function of cholesterol, it needs movement through blood. Because cholesterol does not dissolve in water, it moves through lipoprotein in blood. The lipoproteins can be divided into LDL(Low Density Lipoprotein) and HDL(High Density Lipoprotein) depending on the density. When LDL-cholesterol is too much present in blood, wall of blood vessels will be covered with lipid component. Therefore, it is important to measure value of LDL-cholesterol and diagnose diseases such as cardiovascular disease (CVD), obesity, diabetes, atherosclerosis, hypertension and stroke. In this paper, it shows that measuring the LDL-cholesterol in blood through an immunological method. Paper suggest double bio marker using two types of immunological measuring methods. One is streptolysin O (SLO) recombinant protein that it can specific combine with cholesterol because carboxyl-terminal of SLO has tryptophan-rich. The other is monoclonal antibody about Apolipoproetin B-100 existing in surface of LDL particle. The experimental results show that measuring LDL-cholesterol is dependent concentration and LDL- cholesterol of 2?/ml recognize the value of O.D.630nm

Presenter: Duggan, Jeffrey

BCAP is a negative regulator of myeloid cell development

Jeffrey Duggan, Griffin Gessay, Akshay Krishnamurty, Tobias Hohl, Jessica Hamerman

Dept. of Immunology, Univ. of Washington; Dept. of Immunology,
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B cell adaptor for PI3-kinase (BCAP) is a signaling adaptor protein expressed in cells of the hematopoietic lineage, including the myeloid cell compartment. BCAP has a variety of functions within immune cells, including participating in B cell signaling and homeostasis, NK cell development and function, and macrophage TLR signaling. Here we examined the role of BCAP in myeloid cell homeostasis and development. When examining myeloid cell populations within wild-type (WT) and BCAP-/- mice, we found that, BCAP-/- bone marrow had an increased number of inflammatory monocytes compared to WT bone marrow, whereas splenic populations of inflammatory monocytes, neutrophils, and dendritic cells were similar in number between WT and BCAP-/- mice. Additionally, we observed that mixed bone marrow chimeras generated with a 1:1 ratio of WT and BCAP-/- bone marrow exhibited a skewing towards BCAP-/- monocytes and neutrophils in the bone marrow, blood and spleen, showing a competitive advantage of BCAP-/- myeloid cells. We therefore hypothesized that BCAP regulates myeloid cell development. WT and BCAP-/- bone marrow had similar numbers of myeloid progenitor cells, including the LSK (Lineage-Sca-1+cKit+), CMP (Common Myeloid Progenitor) and GMP (Granulocyte-Macrophage Progenitor) cells. However, in mixed bone marrow chimeras the LSK, CMP, and GMP populations were skewed toward BCAP-/- cells similar to the mature myeloid cells, showing that BCAP-/- progenitors can out-compete their WT counterparts. In an in vitro myeloid colony forming unit assay, sorted BCAP-/- LSK, CMP, and GMP produced more total cells than WT progenitor cells, supporting a cell-intrinsic role of BCAP in regulating myeloid differentiation. To examine the development of inflammatory monocytes from WT and BCAP-/- progenitors in vivo, we used WT and BCAP-/- mice expressing the Ccr2-DTR transgene. After Diphtheria Toxin-mediated depletion of inflammatory monocytes, BCAP-/- mice replenished their peripheral monocyte numbers more rapidly than WT mice. Additionally, when infecting WT and BCAP-/- mice with Listeria monocytogenes, we observed markedly enhanced bacterial clearance in the spleen of BCAP-/- mice compared to WT mice, which correlated with increased numbers of inflammatory monocytes and neutrophils by day 2 post-infection. Together, these data show that BCAP acts as a cell-intrinsic negative regulator of myeloid cell development during both homeostatic and inflammatory settings.

AhR acts independently of Foxp3 to mediate suppression of pancreatic infiltration in NOD mice

Allison Ehrlich, Jamie Pennington, Xisheng Wang, Nancy I. Kerkvliet

Oregon State University

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that has been associated with potent immunosuppression. We have previously reported that suppression of type 1 diabetes by TCDD, a potent AhR ligand, is associated with an increased percentage of Foxp3+ regulatory T cells. Similar associations between AhR activation and increased Foxp3+ cells have been reported for suppression of other autoimmune diseases. Based on the ability of AhR ligands to induce Foxp3+ Tregs, AhR is a potential target for the treatment of autoimmune disease. We have recently discovered a novel, rapidly metabolized AhR ligand, 10-chloro-7H-benzimidazo[2,1-a]benzo[de]Iso-quinolin-7-one (10-Cl-BBQ), that activates AhR in CD4+ T cells in a similar manner to TCDD. Here we show oral treatment with 10-Cl-BBQ is highly effective in suppressing pancreatic islet infiltration throughout 20 weeks of age in the NOD mouse model. When we looked at changes in Foxp3+ cells, a small yet significant increase in the frequency, but not total number, of Foxp3+ cells was observed. This raises the yet unanswered question, is the increase in Foxp3+ Tregs driving AhR-mediated immune suppression? To directly assess the requirement of Foxp3+ cells in AhR induced suppression of islet infiltration, transgenic NOD.Foxp3.DTR (human diphtheria toxin receptor under the control of Foxp3) mice were employed. In the vehicle control group, Foxp3 depletion led to rapid pancreatic islet infiltration. However, treatment with 10-Cl-BBQ suppressed infiltration even in the absence of Foxp3+ cells. These results suggest that AhR functions as a Foxp3-independent transcription factor driving suppression of T cell dependent immune responses.

Presenter: Escalante, Nichole

Exploring the potential role of NLRC3 as a negative regulator of T cell function

Nichole Escalante, Vinicius Motta, Jerry Zhou, Dave Prescott,
Stephen Girardin, Dana Philpott

Department of Immunology, University of Toronto

NLRC3 is an understudied member of the Nod-like receptor family whose function in maintaining immunity is poorly characterized. Previously published work has suggested that NLRC3 may act as a negative regulator of T cell activation however the specific mechanisms through which it acts in T cells remains to be discovered. We have found high mRNA expression of NLRC3 in Natural Killer cells and T cells, with slightly lower expression in B cells and little to undetectable expression in macrophages. In T cells, NLRC3 protein expression was found in the cytoplasm and nucleus. After in vitro T cell receptor (TCR) stimulation, both mRNA and protein NLRC3 expression decreased. NLRC3 knockout CD4+ T cells, however, did not have altered proliferation, activation or IL-2 secretion after in vitro stimulation and analysis of T cells in NLRC3 knockout mice showed normal frequencies of CD4+ AB TCR+, CD8+ ABTCR+ and γΔTCR+ T cells in the thymus, spleen, mesenteric lymph nodes and intestines. Interestingly, NLRC3 knockout mice have decreased colon pathology after Citrobacter Rodentium infection compared to wild type mice. This recent finding suggests that NLRC3 is an important regulator of mucosal immunity in vivo.

Beyond innate immunity: the role of IRF3 in CD4+ T cell function

Nancy Fares-Frederickson, Dennis Otero, Monica Macal, Elina Zuniga, Michael David

University of California, San Diego

Interferon regulatory factor 3 (IRF3) is a transcription factor found in the cytoplasm of most nucleated cells. Upon pathogen recognition, IRF3 becomes activated and translocates to the nucleus where it mediates the production of type I interferon (IFN). Whereas IRF3 has been studied extensively in the context of type I IFN production and innate immunity, its role in adaptive immunity has remained largely unexplored. The objective of our research is to elucidate the role of IRF3 in CD4+ T cell function. Our findings demonstrate that IRF3-deficient CD4+ T cells show robust proliferation in response to TCR engagement, but are compromised in their ability to differentiate into specialized T helper cell subsets-most notably IL-17-producing Th17 cells. As such, CD4+ T cells lacking IRF3 expression fail to induce disease in the T cell transfer model of colitis. These studies reveal a novel, T cell-intrinsic function of IRF3 and extend its role beyond the innate immune response to pathogens.

CD44 controls T cell exhaustion and viral persistence during chronic viral infection

Carrette Florent, Tinoco Roberto, Barraza Monique, Bradley Linda

Sanford Burnham Medical Research Institute

During a chronic viral infection, inhibitory receptors play a crucial role in controlling viral persistence and T cell exhaustion. However, the role of homing molecules in this process has been poorly investigated. Using the chronic LCMV virus model, Clone 13, we found that expression of CD44, a cell surface glycoprotein broadly used to identify activated T cells, dampens antigen specific T cell responses. In CD44-deficient hosts, we observed a significant increase in antigen specific CD4 and CD8 T cells functions with decreased PD-1 expression and a striking increase in multiple cytokine production. T cell accumulation was not due to increased proliferation based on BrdU incorporation, and the increased CD8 T cell response required CD4 T cell help because CD8 T cell exhaustion was maintained in CD4 depleted CD44-deficient mice. Using a bone marrow chimera approach, we found that restricting the CD44 deficiency in the non-hematopoietic compartment was sufficient to reproduce the observations made in the complete CD44 deficient hosts. Finally CD44-deficiency resulted in viral clearance by d15pi. Importantly, treatment of WT mice with a CD44-blocking antibody increased antigen specific CD4 and CD8 T cell recovery and some aspects of T cell function as early as d9pi. Taken together, these results indicate that CD44 is a novel inhibitory receptor that can be targeted to improve T cell response during chronic viral infections.

Presenter: Galimidi, Rachel

Intra-spike crosslinking overcomes antibody evasion by HIV-1

Rachel Galimidi, Joshua S. Klein, Maria S. Politzer, Shiyu Bai, Michael Seaman, Michel Nussenzweig, Pamela J. Bjorkman

California Institute of Technology

Antibodies developed during HIV-1 infection lose efficacy as the virus mutates its envelope spike. We postulated that HIV-1 uses its small spike number to impede bivalent binding of IgGs through inter-spike cross-linking, thus hindering avidity, potent neutralization, and expanding the range of mutations permitting antibody evasion. To test this idea, we engineered antibody based molecules capable of avid binding through intra-spike cross-linking. We used DNA as a "molecular ruler" to measure distances between epitopes on virion-bound envelope and to construct intra-spike cross-linking molecules. Bivalent binding resulted in synergy (>100-fold average increased potency) and shed light on dynamic states of the HIV-1 envelope protein. These results support the hypothesis that low spike densities facilitate antibody evasion and demonstrate that intra-spike cross-linking lowers the concentration of antibodies required for neutralization by up to 2.5 orders of magnitude.

Presenter: Galimidi, Rachel

Overcoming HIV-1 evasion of antibody avidity by intra-spike crosslinking

Rachel Galimidi, Maria S. Politzer, Joshua S. Klein, Shiyu Bai, Michael S. Seaman, Michel C. Nussenzweig, Anthony P. West, Jr., Pamela J. Bjorkman

California Institute of Technology

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Presenter: Gardell, Jennifer

Antigen-specific transfer of CD40L (CD154) from helper T cells to B cells

Jennifer Gardell, David Parker

Oregon Health and Science University

It has been known for decades that the delivery of T cell help for B cells is antigen-specific, MHC-restricted, and depends on CD40L. However the mechanisms by which CD40L, a transmembrane cytokine, is delivered to the T cell surface and engages CD40 on antigen-presenting B cells remains to be determined. Huse et al. (2006 Nat Immunol 7: 247-55) showed that a subset of cytokines is delivered directionally to the point of contact between the T cell and the antigen-presenting cell, also known as the immunological synapse, but CD40L was not among the cytokines investigated in that study. Although CD40L, like other cytokines, is made de novo following T cell activation, Koguchi et al. (2007 Blood 110(7): 2520-7, 2011 J Immunol 187(2): 626-34, 2012 PLoS One 7(2): e31296) showed that CD40L protein is stored in effector and memory CD4 T cells in intracellular vesicles that come to the cell surface rapidly following antigen recognition in sufficient amounts to activate antigen-presenting B cells. It has been thought that when a T cell recognizes an antigen-presenting B cell, CD40L expressed on the T cell surface engages with CD40 on the surface of B cells for a period long enough to lead to productive signaling. Here we show for the first time that CD40L does not remain on the surface of the T cell, but is actually transferred to the B cells. This transfer is absent from bystander B cells that are not presenting antigen, and is only partially dependent upon the presence of CD40 on the antigen-presenting B cells. Choudhuri et al. (2014 Nature 507(7490): 118-23) have recently shown that TCRs are deposited in microvesicles on the antigen-presenting cell at the immunological synapse. Our data suggests that CD40L might be deposited in a similar manner in some type of membrane vesicle on antigen-presenting B cells. This transfer would allow for sustained CD40L-CD40 signaling after a very brief (Allen et al. 2007 Science 315(5811): 528-31, Victora et al. 2010 Cell 143(4): 592-605, Shulman et al. Science 345(6200): 1058-62, Liu et al. 2014 Nature) interaction with helper T cells in the germinal center. If delivery of CD40L is proportional to the amount of antigen on the B cell, transfer of CD40L could be the mechanism by which B cells with higher affinity for antigen following somatic hypermutation are selected in the germinal center reaction, as recently proposed by Dustin (2014 Mol Cell 54(2): 255-62).

Presenter: Gauthier, Kelsey Sivick

Intratumoral Administration of Synthetic STING-Activating Cyclic Dinucleotides: A Promising Approach to Cancer Immunotherapy

Kelsey Sivick Gauthier, Laura Hix Glickman, David B. Kanne, George E. Katibah, Edward Lemmens, Justin J. Leong, Ken Metchette, Thomas W. Dubensky, Jr., and Sarah M. McWhirter

Aduro BioTech

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Presenter: Gillies, Jana

PHLPP1 Restrains Regulatory T Cell Plasticity

Jana Gillies, Scott Patterson, Rosa Garcia, Tianyan Gao, Audrey O'Neill, Alexandra C. Newton, and Megan Levings

University of British Columbia

Regulatory T cells (Tregs) have a reduced capacity to activate the PI3K/Akt pathway downstream of the TCR, and the resulting low activity of Akt is necessary for their development and function. We have shown that the PH-domain leucine-rich-repeat protein phosphatase 1 (PHLPP1), which dephosphorylates Akt, is highly expressed in Tregs and is required for their suppressive function. An important consideration as treg therapy begins to be used clinically is whether tregs will maintain their suppressive phenotype in the inflammatory milieu. We tested whether PHLPP1 had a role in regulating the plasticity of tregs by incubating PHLPP1-/- tregs in Th17 polarizing conditions. We found that without PHLPP1, tregs had a much greater propensity to lose FoxP3 and gain IL-17 expression in comparison to their wild-type counterparts. Pyrosequencing of the TSDR revealed that this loss of FoxP3 was associated with increased TSDR methylation in the PHLPP1-/- tregs. No difference in TSDR methylation was observed in tregs isolated from the thymi of wild type and PHLPP1-/- mice, excluding the possibility that PHLPP1 plays a role in treg development in the thymus. Thus, we tested the hypothesis that phlpp1 prevents plasticity of tregs in the periphery by decreasing their response to Th17-promoting cytokines. However, we found no difference between wild-type and PHLPP1-/- tregs in STAT3 phosphorylation in resonse to IL-6, or SMAD3 phosphorylation in response to TGF-B. We did notice that PHLPP1-/- tregs proliferated more readily than their wild-type counterparts, and that this proliferation was associated with loss of phlpp1 mRNA expression. When we used cell proliferation dye to compare wild type and PHLPP1-/- tregs that had undergone the same amount of cell divisions, we found that they produced equivalent amounts of IL-17 and retained equivalent amounts of FoxP3. Thus, our results show that PHLPP1 plays an indirect role in preventing treg plasticity as it acts by inhibiting proliferation-associated FoxP3 loss. This link between proliferation and FoxP3 loss will be important to consider in the development of treg cell therapies.

Regulation of B cell functions by Integrin alpha 4 during EAE

Simon GLATIGNY, Simon Glatigny, Katie Wagner, Rebekka Duhen, Carlos A Arbelaez, Mohamed Oukka and Estelle Bettelli

Benaroya Research Institute

Experimental autoimmune encephalomyelitis (EAE) is an animal model of multiple sclerosis (MS), mediated by autoreactive myelin-specific T cells. B cells have been implicated in the pathogenesis of MS and EAE. While antigen-activated B cells can differentiate into antibody-secreting plasma cells and serve as potent antigen presenting cells (APCs), other B cell subsets are substantially weaker APCs and may exert anti-inflammatory properties, modulating the effector functions of other immune cells. Furthermore, B cells have been shown to play a regulatory role during EAE as B-cell deficient animals failed to recover and the transfer of a splenic CD1dHigh CD5+ B cell subset promoted the recovery of mice from EAE. Itga4 is important for the entry of lymphocytes in the CNS under inflammatory conditions. However, data from our laboratory showed that elimination or neutralization of Itga4 differentially affect the entry of Th1, Th17 and Treg cells into the CNS. The goal of this study was to determine how Itga4 modulates B cell homing and functions during the course of EAE. Using mice with a specific deletion of Itga4 on B cells (CD19Cre Itga4flox mice), we show that Itga4 control EAE severity since CD19Cre Itga4flox mice develop more severe EAE than control mice. CD19Cre Itga4flox mice have limited number of splenic marginal zone B cells and IL-10 producing B cells infiltrating the CNS compared to control mice. In contrast, Itga4 deficient transitional and follicular splenic B cells induce a stronger T cell response than their Itga4 sufficient counterparts. This study reveals that Itga4 act as a critical regulator of the homing and function of B cell subsets during EAE.

Presenter: Goetz, Christine

Development of a New Mouse/Human FoxP3 Antibody using Rabbit Antibody Technology

Christine Goetz, Ryan Bjordahl, Jody Bonnevier, J.P. Houchins, Cyrus Munshi, & Birte Aggeler

Bio-techne

T regulatory cells (Tregs) play a key role in immune system suppression during autoimmunity and tumor development. Tregs are classified as CD4+CD25+FoxP3+, and exist as either natural Tregs (nTregs) derived from the thymus, or induced Tregs (iTregs) that develop from CD4+ T effector cells in the periphery. Reduction in Treg numbers leads to autoimmunity; this is clearly shown by the scurfy mouse model in which FoxP3 has been deleted, Treg numbers are severely reduced, and mice succumb to autoimmunity early in life. In contrast, Tregs also play a debilitating role in cancer biology when Treg numbers are elevated, and the immune response is dampened allowing cancer cells to evade the immune system. Thus, the correct balance of Tregs is essential for maintaining immune homeostasis. FoxP3 has been widely used as a marker of Tregs, and many antibodies are available on the market that distinguish the Treg population in human or mouse cells. We developed a novel FoxP3 antibody and accompanying Fixation and Permeabilization buffer system that is comparable to current clones, but recognizes both mouse and human FoxP3 equally well, eliminating the need for separate antibodies. The mouse/human FoxP3 antibody was generated using our rabbit antibody technology, which allows for selecting high specificity, high affinity antibodies, lot to lot consistency and detection of both mouse and human nTregs and iTregs. This allows researchers to extend their mouse models into clinical studies without a change in the antibody clone, eliminating a variable and streamlining the process.

Presenter: Goetz, A.

Activation of human dendritic cells in malaria

A. Goetz, Maureen Ty, Ana Rodriguez

NYU School of Medicine

Malaria is characterized by cyclical fevers and high levels of inflammation, and while an early inflammatory response contributes to parasite clearance, excessive and persistent inflammation can lead to severe forms of the disease. At the same time, malaria infections fail to induce durable immunological memory and knowledge of anti-malarial immunity is incomplete. Dendritic cells (DCs) are not only important for the early cytokine responses but also essential for bridging and regulating the innate and adaptive immune responses to pathogenic infections. Very little is known about the role DCs play in the immune response to Plasmodium. Results of studies in both human and animals have been contradictory. To address the role of DCs in malaria immune response we purified human DCs from peripheral blood of healthy donors and co-incubated them with P. falciparum schizonts in vitro. While DCs up-regulated surface expression of the activation markers HLA-DR, CD80, CD86, and CD40, they failed to secrete significant amounts of cytokines, namely IL-8, IL-6, IL-10, TNF, IL-1beta, and IL-12p70. Analysis of the two main human DC sub-populations, plasmacytoid (pDCs) and myeloid DCs (mDCs), revealed that mDCs up-regulated all co-stimulatory markers mentioned above while pDCs only up-regulated HLA-DR. To examine whether P. falciparum-activated human DCs are able to activate and polarize T cells without producing significant amounts of cytokines we will co-incubate DCs with autologous T cells and analyze proliferation and cytokine production. Considering the inadequate immune response to the parasite, Plasmodium could evade the immune system by activating DCs in a suboptimal way, especially in early stages of the disease or low parasitemia.

A role for hypoxia inducible factors in tissue specific anti-viral CD8+ T cell responses

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It has recently been established that components of the transcriptional response to hypoxia can influence the extent to which CD8+ T cells control persistent viral infection and neoplastic growth. These findings suggest that micro-environmental cues can regulate effector CD8+ T cell responses by controlling both differentiation and function. To further investigate this possibility, we used a localized Vaccinia virus (VACV) infection model to further dissect the implication of these signals on polyclonal CD8+ T cell responses. We now demonstrate that a lack of hypoxia-inducible factor (HIF) 1a and 2a expression in CD8+ T cells protects against illness severity, as measured by weight loss, and limits lung pathology. In addition, HIF 1a and 2a expression influences effector and memory subset differentiation and effector molecule expression in VACV specific CD8+ T cells in the lung. Collectively these data demonstrate the importance, and growing need, to better understand how local micro-environmental cues can influence the many transcriptional programs and molecular pathways that regulate CD8+ T cell differentiation. This understanding will inform efforts to better develop efficacious vaccines and therapeutic cell therapies.

Functional Analysis of DNA Sensors in the Interferon Stimulatory DNA (ISD) Pathway

Elizabeth Gray, Dan Stetson

University of Washington

Detection of viral DNA and production of type I interferons (IFNs) are essential for antiviral immunity; on the other hand, inappropriate immune responses to self DNA results in autoimmunity. Intracellular DNA sensing activates the STING-dependent IFN stimulatory DNA (ISD) pathway. The DNA receptors involved in activating this pathway are not fully characterized, although multiple sensors have been proposed. Recent work has defined cyclic GMP-AMP synthase (cGAS) as a key DNA sensor in the ISD pathway, and cGAS-deficient cells have a dramatically impaired IFN response to transfected DNA ligands, DNA viruses, and retroviruses. In addition, the AIM2-like receptors (ALRs) are an evolutionary diverse family of DNA-binding receptors that are also thought to participate in the ISD pathway. Several mouse and human ALRs activate the ISD pathway when overexpressed, and multiple studies have suggested that ALRs are required for an optimal IFN response to various DNA pathogens. On the other hand, others have found that the ISD pathway is intact following ALR knockdown. Thus, while cGAS has clearly emerged as a key DNA sensor in the ISD pathway, the function of the ALRs is still unclear. We have developed novel tools to define the relative contributions of the ALRs and cGAS to the ISD pathway, namely mice lacking all thirteen ALR genes and cGAS-deficient mice. In addition, we have used CRISPR gene targeting to disrupt cGAS and all four human ALRs in human cells. Analysis of IFN induction following transfection of DNA ligands or infection with DNA viruses has revealed that cGAS is absolutely required for activation of the ISD pathway in both mouse and human cells, consistent with recent studies. We are currently examining the ISD pathway in our ALR-deficient mouse and human cells to define how the ALRs function in the innate response to DNA virus infection.

Development of NY-ESO-1 TCR bearing T cells from HSCs is dependent on HLA-A2.1

Eric Gschweng, MacLean Sellars, Michael L Kaufman, Antoni Ribas, Donald B Kohn

University of California, Los Angeles

Engineering hematopoietic stem cells (HSC) for cancer immunotherapy has the potential to provide a life time supply of engineered T cells, overcoming current limitations associated with the use of a differentiated T cell based infusion product. However, the development of T cells from an engineered HSC transplant may be negatively affected when using T cell receptors (TCR) with supraphysiologic affinity. The transplant of human CD34 enriched peripheral blood HSCs transduced with a lentiviral vector encoding an affinity enhanced NY-ESO-1 TCR to immunocompromised mice (NSG) was used to evaluate the development of engineered T cells from HSCs in vivo. We observed an HLA-A2.1 expression dependent effect on the efficiency of development of NY-ESO-1 TCR bearing T cells from HSCs tied to signaling during thymopoiesis. In non A2.1 matched donor and recipient tissue, the majority of NY-ESO-1 bearing cells were halted in the double positive stage of thymopoiesis, with reduced numbers of cells in CD4 and CD8 single positive stages, and few CD8 SP cells in the periphery. Examination of markers of activation in the thymus between the TCR positive and negative fractions revealed a failure of positive selection in this model. Using HLA-A2.1 positive NSG mouse recipients and HLA-A2.1+ donor tissue allowed for positive selection during thymopoiesis, and a greater proportion of peripheral engraftment of CD8 SP NY-ESO-1 TCR bearing T cells. The supraphysiologic affinity of the TCR did not result in negative selection during development, providing support for the use of affinity enhanced TCRs for HSC based engineered immunity.

Thymic stromal lymphopoietin (TSLP) links atopic dermatitis and gastrointestinal allergy

Hongwei Han, Tennille Thelen, Michael Comeau, Steven Ziegler

Benaroya Research Institute

Atopic dermatitis (AD) and food allergy are highly linked, indicating that allergen permeation of the skin may bypass oral tolerance and induce allergy. We show that thymic stromal lymphopoietin (TSLP), an IL-7-related cytokine, initiated an immunological cascade that linked epicutaneous sensitization and gastrointestinal inflammation, resulting in food allergy. Mice sensitized epicutaneously to ovalbumin or peanut in the presence of TSLP developed allergic diarrhea and anaphylaxis following oral antigen challenge. However, IL-33R-deficient mice and mice treated with anti-IL-33 receptor were protected from the development of allergic diarrhea in response to antigen challenge. These data support a role for TSLP and IL-33 in the development of food allergies through epicutaneous sensitization.

CD11c+ and CD4+ cells are important for TSLP-dependent response to hapten in chronic CHS

Angie L. Hertz, Steven F. Ziegler

Benaroya Research Institute

The epithelial-derived cytokine thymic stromal lymphopoieitin (TSLP) has been implicated in the initiation and progression of allergic inflammation through its ability to activate dendritic cells, among other cells. A mouse model of allergic contact dermatitis, also known as contact hypersensitivity (CHS), is a useful tool for dissecting the molecular mechanisms of the allergic response. Our mouse model elicits CHS through sensitization and repeated application of low-dose dinitrofluorobenzene (DNFB). Using various Cre strains coupled to TSLPR-flox to enable deletion of the TSLP receptor on specific subsets of cells we are able to demonstrate a role of TSLP in CHS targeting CD11c+ cells and CD4+ cells. Mice with a deletion of TSLPR on CD4+ cells showed a modest decrease in ear swelling, an indicator of the severity of inflammation. However, mice with a deletion of TSLPR on CD11c+ cells displayed a dramatic decrease in ear swelling, nearly down to the levels of the global TSLPR knockout. These preliminary results indicate that CD11c+ cells may be main driver of TSLP-dependent inflammation in this model of allergic dermatitis.

Characterizing the Murine Immune Response to Corynebacterium pseudotuberculosis

Christopher Hino, Gregory Chisholm, Megan Sumida, Kristi Geiger, Dr.Karen Molinder, Dr.Roberta Pollock

Occidental College

Pigeon Fever is a major infectious equine disease caused by the gram-positive, intracellular 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%. We have developed a mouse model for this disease to examine the roles of different T-cell and antibody responses to infection by C. pseudotuberculosis. BALB/c and C57Bl/6 mice were challenged intradermally with varying doses of live bacteria. Previous experiments have shown that mice mounted a high IgG response after being immunized with bacterial fractionations. However, analysis of serum antibody levels of unvaccinated mice suggests that both mouse strains did not mount a humoral immune response against C. pseudotuberculosis after challenge. Together, these findings suggest that the role of humoral immunity is limited during C. pseudotuberculosis infection and may instead be moderated by a cell-mediated response. This study provides valuable insight into the a strategy by which this intracellular pathogen evades the host immune system and may lead to the design of novel strategies, including the development of effective vaccines, to better control Pigeon Fever.

Presenter: Hoyer, Katrina

CD8 T cell-mediated bone marrow failure

Katrina Hoyer, David M Gravano, Philip D Sanders, Dan Davini, Jennifer O Manilay,

University of California, Merced

Acquired aplastic anemia (AA) is a bone marrow failure disorder characterized by bone marrow hypoplasia and blood pancytopenia and in a majority of patients is autoimmune in nature. Clinical evidence implicates a detrimental role for CD8+ T cells in this disease and a beneficial role of Foxp3+ regulatory T cells (Tregs) in maintaining immune tolerance in the bone marrow. In the IL-2-KO spontaneous autoimmune mouse model, which has a deficit in functional Tregs, we demonstrate a critical role for CD8+ T cells in the development of Th1-mediated aplastic anemia. CD8+ T cells promote hematopoietic stem cell dysfunction and depletion of myeloid lineage progenitor cells, resulting in anemia. Adoptive transfer experiments demonstrate that CD4 cells are required to provide CD8 T cells help to accumulate in the bone marrow and expedite disease progression. Furthermore, we have noted a specific accumulation of TCR VÃŸ6 CD8+ T cells in the bone marrow, raising the possibility that this disease may be due to an antigen-specific immune response.

Presenter: Jackson, Laurice

Enterohemorrhagic E. coli type III-secreted immunomodulatory effectors suppress acute inflammation in vivo and promote lethal disease.

Laurice Jackson, Ilan Rosenshine, John Leong

Tufts University, The Hebrew University of Jerusalem

Not to be placed on the website

Presenter: Johnson, Mark

The Influence of Antigen-Specific TH1 and TH17 Cells in Shaping Neuro-inflammatory Patterns in Patients with Multiple Sclerosis

Mark Johnson, Emily R. Pierson, Mariko Kita, Jane Buckner and Joan M. Goverman

Buckner: Benaroya Research Institute Kita: Virginia Mason
All else: Department of Immunology, University of Washington

Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system (CNS). Although pathology and clinical course vary amongst MS patients, the majority of lesions are found within the brain. However, a small subset of MS patients (2-10%) exhibit inflammation localized to the spinal cord, without extensive involvement of the brain. The mechanisms defining these unique neuro-inflammatory patterns are poorly understood. Using the EAE model, we previously showed that the response to inflammatory, effector T cells is regulated differently in the brain and spinal cord. Specifically, we showed that IL-17 promotes, while IFNγ inhibits, brain localized inflammation through a mechanism involving the recruitment of neutrophils by the ELR chemokine CXCL2. In contrast, IFN promotes CXCL2 expression and subsequent inflammation in the spinal cord. Collectively, these data suggest that the relative abundance and localization of TH17 and TH1 cells are critical determinants in the manifestation of brain and/or spinal cord inflammation in EAE via their disparate effects on CXCL2 induction in these microenvironments. To translate our findings to humans, we investigated the frequency of myelin protein-specific TH1 and TH17 cells in the blood of MS patients with different neuroinflammatory patterns. We hypothesized that patients with lesions only in the brain would have a higher TH17:TH1 ratio compared to patients with predominantly spinal cord lesions. Surprisingly, our results revealed a significant decrease in myelin basic protein (MBP)-specific TH1 cells in patients with predominantly spinal cord lesions compared to patients with lesions only in the brain. A similar trend for reduced numbers of TH1 cells specific for myelin oligodendrocyte glycoprotein (MOG) was observed in spinal cord-predominant patients, as well. In contrast, the majority of patients with brain-only lesions exhibited reduced numbers of MOG-specific TH17 cells compared to patients with predominantly spinal cord lesions. These distinct TH1 and TH17 patterns in response to two myelin proteins in the peripheral blood suggest that the pathogenesis of MS may differ in patients exhibiting brain vs. spinal cord localized lesions.

Engineering High Affinity T-Cell Receptors Specific for Listeria monocytogenes

Deborah Johnson, Bryce E. Anderson, Kemais Ehlers, Stephen P, Persuad, and K. Scott Weber

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CD4+ T cells are central players in immunity to infection and help coordinate both the adaptive and the innate responses to infection. CD4+ T cell activation is dependent on the binding interactions between the T cell receptor (TCR) and peptide-MHC (pepMHC). TCRs have very low affinity for pepMHC; thus we determined to generate pathogen specific high affinity T cell receptors to test if T cell activation and memory cell formation is improved with increased pepMHC affinity. To do this we used two CD4+ T cells, called LLO118 and LLO56, which are specific for the same naturally occurring Listeria monocytogenes epitope. These two T cells have similar affinity for pepMHC and differ by 15 amino acids found mainly in the CDR3 region. Despite these similarities, LLO118 has a stronger primary response while LLO56 has a stronger secondary response. We generated single chain LLO118 and LLO56 TCRs (VB2-linker-VA2) and reasoned that we could use directed evolution to generate stabilized and then high affinity mutants. Single chain LLO118 and LLO56 were fused to the yeast surface protein Aga-2 and error prone PCR was used to generate mutagenic libraries. Stabilized single chain TCRs were selected for using biotinylated VB2 and VA2 antibodies and anti-biotin beads. LLO118 and LLO56 stabilized clones were then used as templates to generate affinity libraries using site directed mutagenesis of the CDR3 region. We have sorted the LLO118 library with pepMHC tetramers (LLO190-201) and have isolated clones with improved binding affinity. Generation of high affinity pathogen specific TCRs should increase our understanding of the relationship between TCR:pepMHC avidity and T cell activation. These TCRs will also aid in attempts to identify the characteristics of CD4+ T cells needed for effective memory cell generation and should provide useful insights for improving vaccine design and immunotherapies.

Autophagy protects monocytes from Wolbachia heat shock protein 60 (rWmhsp60) induced apoptosis and senescence

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Monocyte dysfunction by filarial antigens have been postulated as major mechanism underlying immune evasion following hypo responsiveness during patent lymphatic filariasis. Recent studies have initiated a paradigm shift to comprehend the immunological interactions of Wolbachia and its antigens in inflammation, apoptosis in monocytes, lymphocyte anergy, etc. Here we showed that rWmhsp60 (recombinant Wolbachia heat shock protein 60) interacts with TLR-4 and induces apoptosis in monocytes of endemic normal, but not in chronic patients. Higher levels of reactive oxygen species (ROS) induced following TLR-4 stimulation resulted in loss of mitochondrial membrane potential and caspase cascade activation, which are the plausible reason for apoptosis. Furthermore, release in ROS owing to TLR-4 signaling resulted in the activation of NF-κB p65 nuclear translocation which lead to inflammation and apoptosis via TNF-receptor pathway following the increase in IL-6 and TNF-a levels. Here for the first time, we report that in addition to apoptosis, rWmhsp60 a possible antigen in filarial pathogenesis also induce molecular senescence in monocytes. Targeting TLR-4 therefore presents a promising candidate for treating rWmhsp60 induced apoptosis and senescence. Strikingly, induction of autophagy by rapamycin, detains TLR-4 in late endosomes and subverts TLR-4-rWmhsp60 interaction thus protecting TLR-4 mediated apoptosis and senescence. Also, rapamycin induced monocytes were unresponsive to rWmhsp60 and triggered lymphocyte activation and proliferation following PHA stimulation. With the perspective of the evidences put forward, targeting inherent autophagic degradation pathway may provide perfect catalyst to herald a potential area of research for the development of inherent methods to treat filariasis and other inflammatory conditions.

B cell responses to vaccines in the aged

Senthil Kannan, Hildegund C.J. Ertl, Raj Kurupati, Larissa Haut, Ken E. Schmader, Susan Doyle,

University of Pennsylvania , The Wistar Institute

Vaccines have prevented millions of deaths ranging from primarily pediatric diseases like Rubella to more diverse ones like Influenza, Measles and Shingles. However, the efficiency of vaccines drops sharply with age, and thus leaves our aging population at a disadvantage. This decrease in immune function-immunosenescence- is thus a vital area of interest. My research addresses immunosenescence in mice and humans, specifically looking at B cells and Antibody Secreting Cells and their responses to the inactivated Influenza vaccine. This has resulted in a number of interesting findings. A number of differences were observed by multicolor flow cytometry between young and aged mice including differences in expression of immune regulators like Programmed Death -1 (PD-1) and its ligand Programmed Death Ligand-1 (PD-L1). Metabolic differences were also observed, including higher levels of basal mitochondrial respiration in the young mice. We conducted similar studies in young and aged humans receiving the inactivated influenza vaccine, and observed a role of the B and T Lymphocyte Attenuator (BTLA) - Herpes Virus Entry Mediator (HVEM) pathway in generating antibody responses, and ASC numbers.

Histone Deacetylase 7 Controls the Thymic Development of iNKT Cells and Other Innate-like Lymphocytes

Herbert Kasler, Intelly S. Lee, Hyung W. Lim, Eric Verdin

Gladstone Institute of Virology and Immunology,
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The growing family of innate-like tissue-resident lymphocytes, comprising several types of agonist-selected T cells as well as innate lymphoid cells, constitutes a vital first line of defense at mucosal barriers. These cells are particularly abundant in the digestive tract and associated organs, which constitute a crucial interface with the potentially dangerous microbiota of the gut lumen. There, they mount rapid effector responses in response to a variety of stimuli, in a manner more characteristic of innate immune cells than T cells. We have discovered that Histone Deacetylase 7 (HDAC7), a class IIa HDAC that we previously found to regulate TCR-dependent apoptosis during negative thymic selection of conventional T cells, also controls the development of invariant natural killer T cells (iNKT cells), a CD1d-restricted population of lymphocytes expressing an invariant Va14Ja18 TCR. These and other related cell types require expression of the BTB-POZ family protein PLZF, which has been shown previously to interact with HDAC7, for their development and acquisition of innate-like effector function. Mice expressing a dominant gain-of-function mutant of HDAC7 (HDAC7-P) in the thymus completely lack iNKT cells, while positive selection of conventional T cells in unimpaired. Conversely, thymocytes lacking HDAC7 acquire rapid effector function and the expression of memory markers constitutively, a finding that strongly mirrors the phenotype observed due to enforced thymic expression of PLZF. Moreover, we have found that HDAC7 and PLZF regulate highly overlapping sets of genes in thymocytes that are highly relevant to the development of innate-like function in lymphocytes. Lastly, mice expressing a HDAC7-P in the thymus, despite exhibiting a global defect in negative thymic selection and autoantibodies to a wide array of tissue-specific antigens, only develop autoimmunity in the anatomic compartments normally most populated by innate-like lymphocytes. Our findings establish HDAC7 as a key gatekeeper of the innate-like lymphocyte effector program via modulation of PLZF activity, and also provide potentially important insights into the relationship between innate-like effector function and the maintenance of immune self-tolerance.

Presenter: Khan, Shahzada

ECD8 T cell response is delayed and dampened upon vaginal versus systemic LCMV infection

Shahzada Khan, Ben Cohn, Miles Oliva, Timothy C Borbet, Ashley Hughey, Emily Deal, Martin Trepcar, Elze Rackaytete, Erik Woodruff, Julie Luong, and Shomyseh Sanjabi

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We currently lack vaccines against many sexually transmitted pathogens, and we know little about the mechanisms of how effective antigen-specific CD8+ memory T cells are induced and maintained in the female reproductive tract (FRT). FRT has an immunologically tolerogenic environment that is equipped to tolerate allogeneic sperm and support the growth of semi-allogenic fetus, but is also the portal of entry for many sexually transmitted pathogens. Our goal is to understand the immune cell populations and mechanisms that orchestrate the fine balance between this tolerogenic environment and the inflammation that is induced in response to vaginal viral infections. We have established a vaginal lymphocytic choriomeningitis virus (LCMV) infection model, allowing us to compare the mucosal versus systemic immune response that is elicited against this pathogen. We have found that intravaginal LCMV infection leads to a delayed and dampened CD8 cytotoxic T lymphocyte (CTL) response compared with systemic infection. The delayed priming of CD8 T cells after intravaginal infection is due to the amount of time that LCMV takes to reach the iliac lymph node (iLN) from the lower FRT. Inoculation of a higher viral dose resulted in a higher CTL response, but could not overcome the delay in CD8 T cell priming. The poor magnitude in the CTL response after vaginal infection was preceded by a dampened type I interferon response, and a reduced recruitment and activation of CD11b+ dendritic cells (DCs) in the FRT and the draining lymph nodes. Priming and activation of CD8 T cells positively correlated with viral clearance in the LNs but not in the lower FRT during the first week of infection. These observations describe a fundamental “defect” in the FRT to mount timely and effective CTL responses against vaginal viral infections, which may provide a “window of opportunity” to sexually transmitted pathogens. Identifying the cell populations and/or tolerogenic mechanisms involved in dampening the CTL response in the FRT would inform future studies for better therapeutic/preventive interventions.

Presenter: Koch, Meghan

TLR-induced anti-commensal IgG antibodies regulate gut immune homeostasis

Meghan Koch, Gabrielle Reiner, Kyler Lugo, Thaddeus Sehar, William Ludington, Gregory Barton

University of California, Berkeley

Maternal antibodies have been suggested to be important for establishing intestinal immune homeostasis in developing offspring, yet the mechanisms by which maternal antibodies achieve this remain unclear. We analyzed the gut immune response in mice born to antibody-deficient dams and found that maternal antibodies regulate the activation of mucosal CD4+ T cells. Interestingly, maternal IgA, the dominant mucosal antibody isotype, was not necessary for this regulation. As such, we developed an unbiased, flow cytometric based assay to assess the microbiota-specific antibody response, thereby allowing us to investigate if other antibody isotypes are important for enforcing intestinal immune homeostasis. Unexpectedly, we found that mice generate a robust anti-commensal IgG2b- and IgG3- response. This commensal-specific IgG response occurs through a pathway independent of T cells, yet dependent on signaling through TLR2 and TLR4. These TLR-dependent anti-commensal IgG antibodies are transmitted from mothers to their offspring. There, they function to regulate T follicular helper responses and subsequently dampen germinal center B cell responses. This work reveals a feedback loop whereby T cell-independent antibody responses function to regulate mucosal CD4+ T cell responses and thereby establish equilibrium between the host and its resident microbiota.

Presenter: Kovar, Marek

IL-2/anti-IL-2 complexes potently stimulate expansion of activated OT-I CD8+ T cells but not activated OT-II CD4+ T cells

Marek Kovar, Barbora Tomalova, Jakub Tomala, Blanka Rihova

Laboratory of Tumor Immunology, Institute of Microbiology AS CR, Prague, Czech Republic

The in vivo biological activity of IL-2 can be dramatically increased by complexing IL-2 with anti-IL-2 mAb. Moreover, these IL-2 immunocomplexes have selective stimulatory activity depending on anti-IL-2 mAb used. IL-2/S4B6 immunocomplexes (henceforth IL-2icS4) are highly stimulatory for memory CD8+T and NK cells (i.e. CD122high populations). They have also moderate stimulatory activity for Treg cells. Conversely, IL-2/JES6-1 mAb immunocomplexes (henceforth IL-2icJ6) have no effect on CD122high cell populations, but they potently expand Treg cells (i.e. CD25high population). Interestingly, both IL-2ic are very potent in expanding activated CD8+T cells in vivo. We demonstrated that IL-2icS4 and IL-2icJ6 exhibited very high activity to drive expansion (~ 100-200 times within 4 days) of activated OT-I CD8+ T cells by using adoptive transfer of CFSE-labeled purified CD8+ T cells from OT-I transgenic mice into congeneic Ly5.1+ mice strain followed by injection of SIINFEKL peptide. Free IL-2 administered at the same dosage (1.5 μg/dose) had negligible effect. IL-2icJ6 were even slightly more potent than IL-2icS4 in terms of expansion of activated OT-I CD8+ T cells. Next, we have done similar experiment but instead of OT-I system we used OT-II system in order to evaluate the potential of IL-2ic to expand activated CD4+ T cells. Unexpectedly, we have found that both kinds of IL-2ic have extremely poor stimulatory activity to drive expansion of activated CD4+ T cells in vivo. IL-2icJ6 expanded activated CD4+ T cells less than 3 times and IL-2icS4 showed no expansion at all. To figure out whether OT-II CD4+ T cells do not possess any inherited limitation to vigorously proliferate, we have compared proliferation of OT-I CD8+ T cells and OT-II CD4+ T cells induced by syngeneic DCs loaded with respective peptide in vitro. However, both cell subsets showed comparable proliferation. Thus, we speculate whether activated CD4+ T cells are much less sensitive to stimulatory activity of IL-2ic in vivo.
Acknowledgement: This work was supported by Czech Science Foundation grant P301/11/0325, 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: Krishnamurty, Akshay

Defining the role of Plasmodium-specific memory B cells

Akshay Krishnamurty, 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, symptomatic erythrocyte-residing stage. A lack of understanding of the immune mechanisms associated with protection has hindered the development of an effective vaccine. B cells are known to be critical mediators of immunity against the blood stage of Plasmodium infection but little is known about the development or function of protective malaria-specific memory B cells. We have generated a novel B cell tetramer reagent to identify B cells specific for the C-terminal region of Merozoite Surface Protein 1 (MSP1), a parasite surface protein expressed in the blood-stage of infection. Using this tetramer and a magnetic bead-based enrichment method, we can track and analyze MSP1-specific B cells during blood stage infection in a well-characterized rodent model of malaria, Plasmodium chabaudi chabaudi. This approach allows us to directly assess how Plasmodium-specific memory B cells develop during blood stage infection and subsequently respond during a secondary infection. MSP1-specific B cells expand within days of infection and as the parasite is cleared and germinal centers form, we observe the formation of a MSP1-specific memory B cell population. As the GC reaction eventually terminates, remarkably persisting for at least 165 days post infection, a stable population of MSP1-specific memory B cells continues to persist at high levels up to 265 days post infection, made up of IgG+ MBCs and more recently described IgM+ MBCs. We are currently assessing the specific functions of these memory populations in an effort to understand how memory B cells contribute to protection during a secondary Plasmodium infection. These studies represent the first glimpse of the endogenous memory B cell response to Plasmodium with the hopes of defining what constitutes optimally protective anti-malarial B cell immunity.

Differential regulation of TGF-[b] activation and Th17 cell generation by DC and macrophage subpopulations

David Kugler, Helena Paidassi, Fiona Raso, Mridu Acharya, Adam Lacy-Hulbert

Benaroya Research Institute

Th17 cells play important protective roles during bacterial and fungal infection at mucosal surfaces, but also contribute to inflammatory and autoimmune disorders. How DCs and other APCs regulate the differentiation and maintenance of Th17 cells and related T cell subsets to achieve this delicate balance between defense and damage remains poorly understood. Differentiation of both Th17 cells and closely related peripheral Tregs relies on TGF-B signaling. We have shown that this is critically dependent on expression of integrin Av B8 by DCs, which activates latent TGF-B for signaling to interacting T cells. Consequently, mice lacking Av in DCs have impaired peripheral Tregs and develop spontaneous colitis, but also very few Th17 cells and are protected from Th17-mediated autoimmunity. While DC Av was necessary for development of both cell types, it is unclear if the same population of DCs supports both fates. To clarify this we are studying the role of DC Av during infection with Citrobacter rodentium, an intestinal pathogen that stimulates strong Th17 immunity. We find that deletion of Av using CD11c-CRE and LysM-CRE lines, which delete predominantly in DCs and macrophages respectively, resulted in distinct effects on Treg and Th17 differentiation. Most notably, DC-specific deletion led to a profound loss of RORγT+ IL-17A+ T cells and intestinal Tregs, which was less pronounced in macrophage-specific Av knockouts. In contrast, both mouse strains showed an increase in IL17A+ IFN-γ+ T cells, which have been implicated in Th17-mediated pathology. Our data suggest that distinct populations of DCs are responsible for generation of IL17A+ and IL17A+ IFN-γ+ Th17 as well as peripheral Treg cells. In ongoing experiments, we are determining the identity of these subpopulations and the signals that induce their expression of AvB8. This project is supported by NIH grant DK093695 to ALH

Presenter: Kuhns, Michael

Evidence for a pivot point in TCR-CD3 triggering

Michael Kuhns

University of Arizona

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Enhanced Th2 cell differentiation and function without NADPH oxidase 2 (Nox2)

Bo-In Kwon, Seung-Hyo Lee

KAIST

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Presenter: Kwun, Jean

Costimulation Blockade and Bortezomib to desensitize Rhesus macaque and prolong renal allograft survival

Jean Kwun, Christopher Burghuber, Eugenia Page, Adriana Gibby, Neal Iwakoshi, and Stuart Knechtle

Duke University

[Background] Preformed donor specific antibody (DSA) due to prior sensitization affects a significant population of transplant patients, portending a poorer prognosis in short- and long-term graft survival. We investigated the effect of proteasome inhibitor (Bortezomib) and costimulation blockades (COB; Belatacept and 2C10, the latter an anti-CD40L mAb) on DSA and plasma cells in pre-sensitized rhesus macaques in order to develop a sustainable desensitization regimen. [Method] All rhesus macaques were sensitized by a full MHC class I mismatched skin graft (~ 2cm diameter). Grafts all rejected within 2 weeks without treatment. Animals received Bortezomib (N=3, 1.33mg/m^2 or Bortezomib with COBs (N=3) twice a week for four weeks when their DSA level reached plateau. [Results] Bortezomib treatment reduced bone marrow plasma cells (IgD-CD20-CD38+CD19+) but follicular helper T cells (Tfh; CD4+ICOS+PD-1high) were significantly increased. In accordance with this, DSA was not significantly reduced by Bortezomib treatment alone. In order to suppress Tfh cell induction, COBs, Belatacept and 2C10, were added to Bortezomib. The combination of COB and bortezomib significantly reduced BM plasma cells (9.60±1.22 vs. 25.66±12.27%; $p<0.05$), serum DSA levels, and LN Tfh cells compared to Bortezomib alone or COB alone. To confirm the desensitization effect, kidney transplantation was performed. Renal allografts without desensitization showed accelerated rejection (N=5, MST=3.6±2.7d). In contrast, desensitization with Bortezomib/Belatacept/2C10 treatment dramatically prolonged graft survival (N=2, MST>28±8.4d; $p<0.05$) with no signs of rejection. [Conclusion] Desensitization with Bortezomib alone moderately reduces both plasma cells and DSA but induces germinal center responses reflected by Tfh cell populations. However, targeting costimulation signals via Belatacept and 2C10 (anti-CD40L) in conjunction with Bortezomib profoundly reduced Tfh cell populations, plasma cell number, and serum DSA. Desensitization with Bortezomib/Belatacept/2C10 significantly prolonged renal allograft survival compared to control groups without desensitization.

Presenter: Lai, Jen-Feng

Crucial role for CD11c+ interstitial macrophages in the development of TSLP-mediated allergic airway inflammation

Jen-Feng Lai, Lucas J. Thompson, Steven F. Ziegler

Benaroya Research Institute

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Presenter: Lau, Laura

DNA tumor virus oncogenes antagonize the interferon stimulatory DNA pathway

Laura Lau, Dan Stetson

University of Washington

Not to be placed on the website

Presenter: Lee, Intelly

Roles of HDAC4 in T-Cell Development & Effector Function

Intelly Lee, Herbert Kasler, Hyungwook Lim, Eric Verdin

University of California - San Francisco

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Presenter: Lim, Hyungwook

SIRT1 deacetylates ROR[g]t and enhances Th17 cell generation

Hyungwook Lim, Seung Goo Kang, Jae Kyu Ryu, Birgit Schilling, Mingjian Fei, Intelly S. Lee, Amanuel Kehasse, Kotaro Shirakawa, Masaru Yokoyama, Martina Schnolzer, Herbert G. Kasler, Hye-Sook Kwon, Bradford W. Gibson, Hironori Sato, Katerina Akassoglou, Changchun Xiao, Dan R. Littman, Melanie Ott, and Eric Verdin

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The balance of effector and regulatory T cell function, dependent on multiple signals and epigenetic regulators, is critical to immune self-tolerance. Dysregulation of T helper 17 (Th17) effector cells is associated with multiple autoimmune diseases, including multiple sclerosis. Here we report that Sirtuin 1 (SIRT1), a protein deacetylase previously reported to have an anti-inflammatory function, in fact promotes autoimmunity by deacetylating RORγt, the signature transcription factor of Th17 cells. SIRT1 increases RORγt transcriptional activity, enhancing Th17 cell generation and function. Both T cell-specific Sirt1 deletion and treatment with pharmacologic SIRT1 inhibitors suppress Th17 differentiation and are protective in a mouse model of multiple sclerosis. Moreover, analysis of infiltrating cell populations during disease induction in mixed hematopoietic chimeras shows a marked bias against Sirt1-deficient Th17 cells. These findings reveal an unexpected pro-inflammatory role of SIRT1 and importantly support the possible therapeutic use of SIRT1 inhibitors against autoimmunity.

Lymphatic vessel expansion and function during dermal vaccinia viral infection

Amanda Lund, Christopher Loo, Jeffrey Nolz

Oregon Health & Science University

While lymphatic vessels are appreciated for their role in fluid drainage and cell trafficking, their active role in the regulation of local immunity is just beginning to be appreciated. Recent work has demonstrated that lymphatic endothelial cells exhibit immunomodulatory functions including the promiscuous expression of peripheral tissue antigens, cross-presentation of lymph-borne antigens and inhibition of T cell and DC function both at steady state and in experimental cancer models. How these vessels and their functional drainage specifically influence the immune response in an active model of acute inflammation, however, remains unknown. In this work we test the hypothesis that remodeling of the lymphatic vasculature during local dermal infection with vaccinia virus defines local immune activity. This suggestion that lymphatic vessel function is linked to the immune status of the tissue it drains is consistent with clinical observations of secondary lymphedema in human herpes infections and increased susceptibility to cutaneous immune pathology associated with sites of lymph stasis. Using dermal infection with vaccinia virus expressing the LCMV epitope gp33, we demonstrate rapid lymphatic hyperplasia and dynamic regulation of fluid drainage from the affected tissue. This lymphangiogenic response is associated with simultaneous angiogenesis and rapid activation of both endothelium as defined by their expression of cellular trafficking molecules (e.g P and E selectin, and ICAM-1). Interestingly, coincident with the infiltration of the infected tissue with T lymphocytes, lymphatic endothelial cells adopt a new pattern of cell surface expression including the expression of negative regulatory molecules (i.e. PD-L1) and MHCII. Expression of these molecules by lymphatic endothelium was dependent upon IFNγ signaling provided at least in part, but not completely, by infiltrating CD4+ T cells. These results begin to demonstrate the potential dual role of the lymphatic vasculature in both the inductive and resolution phases of the immune response against viral infection. Understanding mechanisms of tissue resident immune regulation by the endothelial compartments will provide insight into the way in which cellular trafficking patterns (both entry and exit) regulate regional immunity in the skin.

Thymic defects, lymphopenia and elevated double-negative T cells occur before clinical arthritis in Inherited Inflamed Joints (IIJ) mice

Eileen McMahon McQuade, Jake A. West, David B. Thompson, Katherine M. Shaum, Matthew L. Combs

Department of Biology, Westmont College

The Inherited Inflamed Joints (IIJ) mouse is a novel inbred strain in which approximately a third spontaneously develop an inflammatory arthritis with serological and histological similarities to rheumatoid arthritis in humans. A high percentage of double-negative (DN; CD3+, TCRB+, CD4-, CD8-, B220-, DX5-) T cells can also be found in the spleen and lymph nodes of arthritic (AR) IIJ mice and not in their non-arthritic (NAR) littermates. The goal of our project was to further examine the T cell compartment to determine its possible role in disease initiation. T helper cell subsets were examined using a CBA assay. Upon in vitro activation, splenocytes from AR-IIJ mice produced higher levels of IFN-γ, IL-17A, IL-10, TNF-A, and IL-6 compared to NAR littermates. While Th1 and Th17 cells were therefore present during active arthritis, we wanted to identify early cellular events and subsequently examined the peripheral blood and thymus before disease development. We found that DN T cells were present in the peripheral blood before clinical symptoms occurred, and that they were 100% predictive of future arthritis ("pre-AR" IIJ mice). In both AR and pre-AR IIJ mice, a higher percentage of DN T cells were also found in the thymus and thymi were reduced in size, indicating possible defects in T cell development. Upon closer analysis, a T cell lymphopenia was clearly evident in the peripheral blood with reductions in both CD4 and CD8 subsets. Finally, NAR IIJ T cells adoptively transferred into pre-AR mice readily proliferated and were able to delay arthritic onset and mitigate clinical severity. Therefore, we currently hypothesize that lymphopenia and homeostatic expansion might be driving arthritis in this model. The root cause of the thymic defect is still under investigation.

microRNA-132 regulates hematopoietic stem cell function and survival through FOXO3

Arnav Mehta, David Baltimore

California Institute of Technology

A complex network of interactions tightly regulates hematopoiesis to ensure balanced and appropriate output of blood cells, both under normal and stressful conditions. This network includes a novel class of RNA molecules, called microRNAs, that do not code for proteins, but instead negatively regulate the expression of genes. These molecules serve as "fine-tuners" of gene expression, and when dysregulated, can drastically alter the balance of hematopoiesis, potentially leading to cancer. We have identified microRNA-132, an LPS-inducible microRNA in macrophages, to be enriched in certain hematopoietic compartments. Importantly, ectopic expression of microRNA-132 leads to proliferation of hematopoietic stem cells (HSCs) and early progenitors, with eventual exhaustion and extra medullary hematopoiesis. Deletion of microRNA-132 leads to increased survival of HSCs, especially in conditions of continuous inflammatory stress. We have determined that this phenotype is primarily mediated by the microRNA-132 target FOXO3, a key transcription factor in regulating HSC proliferation and protective autophagy of aged HSCs. We have thus identified microRNA-132 as an important regulator of HSC self-renewal and function, with therapeutic implications in hematopoietic aging and cancer.

CCL21 defect impairs dendritic cell trafficking in SAMP1/YitFc mice

Zbigniew Mikulski, Rebecca Johnson, Iftach Shaked, Gisen Kim, Heba Nowyhed, Wendy Goodman, Grzegorz Chodaczek, Theresa Pizarro, Fabio Cominelli, Klaus Ley

La Jolla Institute

SAMP1/YitFc (SAMP) mice develop chronic ileitis similar to Crohn's disease. The disease mechanism is unknown but thought to involve adaptive and innate immune responses. Here, we discovered that the chemokine CCL21, a ligand for CCR7, is almost completely absent in mesenteric lymphatics and other tissues of SAMP mice. Lymphatic CCL21 is known to be required for dendritic cell (DC) trafficking. Absence of CCL21 results in a severe defect of CD11b+CD103+ DC migration from the ileal lamina propria to the mesenteric lymph nodes (MLN), similar to the defect seen in CCR7-deficient mice. The ability of DCs to produce retinoic acid supporting regulatory T cells (Tregs) is also drastically reduced in SAMP mice. In young mice, the defects in CCL21 expression and DC trafficking preceded the clinical manifestation of ileitis. As a therapeutic intervention, we mobilized DCs by oral treatment with the TLR7 ligand R848, which increased Tregs in MLN and dramatically improved disease scores. Thus, absence of CCL21 expression represents a major pathogenic defect contributing to ileitis in SAMP mice. Our data suggest that therapies aimed at improved DC trafficking might be useful in patients with Crohn's disease.

Mesenchymal stem cell treatment modulates the CD8 T cell response in feline chronic gingivostomatitis

Emily Mills Ko, Emily Mills Ko, Boaz Arzi, Amir Kol, Naomi Walker, Kaitlin Clark, Dori Borjesson

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Mesenchymal stem cells (MSCs) have potent immunomodulatory and trophic properties. Feline chronic gingivostomatitis (FCGS), a naturally occurring, chronic oral inflammatory disease marked by severe lymphoplasmacytic inflammation of the oral mucosa and gingiva. Intravenous (IV) administration of autologous MSCs modulates the immune response, reduces disease severity, and regenerates the oral mucosa in cats with non-responsive FCGS. Of the 8 cats treated with 2 doses each, 3 were completely cured, 3 had significant clinical improvement and 2 did not respond to treatment. Cats that were completely cured had high blood CD8+ T cell numbers at presentation with marked clinical improvement associated with a correction of elevated CD8+ T cell numbers at 6 months post MSC therapy ($p<0.05$). We hypothesize that MSCs regulate the proliferation and cytotoxicity of CD8+ T cells. We also hypothesize that MSCs reduce the expression by CD4 T cells of key signals required to maintain memory populations which sustain the CD8+ T cell response over time. MSC administration to cats with spontaneous FCGS may serve as a novel animal model to better understand MSC interactions with the immune system in vivo, and to translate regenerative medicine therapies to treat chronic human oral inflammatory disorders.

Presenter: Mohar, Isaac

Parallel isolation of non-parenchymal cells from the mouse liver

Isaac Mohar (1,2), Katherine J. Brempelis (1), Sara A. Murray (1), I. Nicholas Crispe (1)

University of Washington (1), Gradient (2)

Intrahepatic infection or tissue damage can significantly alter the liver, where recruited leukocytes are important determinants of immunity and recovery. In a healthy liver, the principle cell types are hepatocytes, liver sinusoidal endothelial cells (LSEC), Kupffer cells, and hepatic stellate cells (HSC). Each of these cell types appears to uniquely participate in leukocyte recruitment and immune regulation. Thus, dissecting the dynamics of each cell type can provide important information about the pathology and immunology of liver tissue. This paper describes a relatively rapid and efficient method for isolating mouse liver non-parenchymal cells in parallel (e.g., LSEC, Kupffer cells, natural killer (NK) and NK-T cells, dendritic cells, CD4+ and CD8+ T cells, and quiescent HSC). In addition to the NPCs, peripheral blood, intact liver tissue, and hepatocytes can be collected. The basic method entails in situ perfusion via the portal vein in order to maximize liver digestion. Next, differential and gradient centrifugation enriches NPCs. The NPCs can then be analyzed or sorted into highly enriched cell types using flow cytometry. The isolated cells are suitable for flow cytometry, protein, and mRNA analyses, as well as primary culture. As an example application, we present a detailed method to study liver-stage malaria infection. The data illustrate qualitative and quantitative differences in the leukocyte response to P. yoelii rodent malaria infection compared with that of a P. yoelii vaccine strain.

A Crohn's disease variant in Atg16l1 enhances its degradation by caspase 3

Aditya Murthy, Yun Li, Ivan Peng, Mike Reichelt, Anand Kumar Katakam, Rajkumar Noubade, Merone Roose-Girma, Jason DeVoss, Lauri Diehl, Robert R. Graham, Menno van Lookeren Campagne

Genentech, Inc.

Crohn's disease is a debilitating inflammatory bowel disease (IBD) that can involve the entire digestive tract. A single-nucleotide polymorphism (SNP) encoding a missense variant in the autophagy gene ATG16L1 (rs2241880, Thr300Ala) is strongly associated with the incidence of Crohn's disease. Numerous studies have demonstrated the effect of ATG16L1 deletion or deficiency; however, the molecular consequences of the Thr300Ala (T300A) variant remains unknown. Here we show that amino acids 296-299 constitute a caspase cleavage motif in ATG16L1 and that the T300A variant (T316A in mice) significantly increases ATG16L1 sensitization to caspase-3-mediated processing. We observed that death-receptor activation or starvation-induced metabolic stress in human and murine macrophages increased degradation of the T300A or T316A variants of ATG16L1, respectively, resulting in diminished autophagy. Knock-in mice harbouring the T316A variant showed defective clearance of the ileal pathogen Yersinia enterocolitica and an elevated inflammatory cytokine response. In turn, deletion of the caspase-3-encoding gene, Casp3, or elimination of the caspase cleavage site by site-directed mutagenesis rescued starvation-induced autophagy and pathogen clearance, respectively. These findings demonstrate that caspase 3 activation in the presence of a common risk allele leads to accelerated degradation of ATG16L1, placing cellular stress, apoptotic stimuli and impaired autophagy in a unified pathway that predisposes to Crohn's disease. Murthy, A. et al. Nature. 2014 Feb 27;506(7489):456-62. doi: 10.1038/nature13044. PMID: 24553140

Cell-type specific control of immune responses mediated by inflamamsomes in vivo

Randilea Nichols, Jakob von Moltke, and Russell E. Vance

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Upon pathogen recognition, certain cytosolic nucleotide-binding domain, leucine-rich repeat containing proteins (NLRs) form multi-protein complexes called inflammasomes, which activate CASPASE-1. CASP1 initiates inflammation by processing IL-1beta and -18 into their active forms, and by inducing a rapid, lytic cell death called pyroptosis. The NLRC4 inflammasome responds to the cytosolic presence of specific bacterial proteins, such as flagellin, via NAIPs. A fundamental question is whether and how activation of innate immune responses triggers pathogen-specific adaptive immune responses. We seek to test the hypothesis that cell-type-specific activation of the inflammasome is sufficient to induce inflammation and adaptive immune responses in vivo. To test our hypothesis, we are using a novel â€˜knock-inâ€™ mouse strain, which can selectively activate the NLRC4 inflammasome. This mouse contains a gene encoding chicken ovalbumin (OVA) fused to the C-terminal 166 amino acids of Legionella pneumophila flagellin (Fla). The OVA-Fla fusion is inserted into the constitutively expressed Rosa26 locus, downstream of a loxP-flanked transcriptional STOP cassette, which prevents OVA-Fla expression unless Cre recombinase excises the STOP cassette. OVA-Fla:LysM-Cre mice, which express the OVA-Fla fusion protein in neutrophils, monocytes, and macrophages, display a visible inflammatory phenotype at 4-5 weeks of age. OVA-Fla:LysM-Cre mice suffer from anemia, increased serum cytokines/chemokines, and cellular changes in the lymph nodes and spleen. Histology shows bone erosion, neutrophil infiltration, and swelling of the tibiotarsal joint, consistent with an arthritis-like disease. Our preliminary data indicate extensive NLRC4-dependent inflammatory disease in mice expressing OVA-Fla in LysM-positive cells.

Differential Expression of Core 2 O-Glycans and Trafficking of Diverse Memory CD8+ T cell Populations

Jeffrey Nolz, Tahsin Khan, Augustus Kilgore

Oregon Health and Science University

Following successful vaccination or pathogen clearance, recently activated CD8+ T cells that survive contraction differentiate into long-lived memory populations and provide host protection against re-infection. We have previously demonstrated that memory CD8+ T cells acquire the capacity to generate core 2 O-glycans in an antigen-independent manner, a post-translational modification required for binding to P- and E-selectin. In addition, we have shown that de novo synthesis of core 2 O-glycans is required for memory CD8+ T cell trafficking to inflamed tissues. However, the molecular mechanisms that regulate expression of core 2 O-glycans in diverse memory CD8+ T cell populations remain largely unknown. Herein, we show that primary memory CD8+ T cells (stimulated with antigen once) traffic to skin infected with Vaccinia virus much more efficiently than tertiary memory CD8+ T cells (stimulated with antigen three times). Trafficking to inflamed skin occurred in an antigen-independent manner, but memory CD8+ T cell-mediated elimination of virus required cognate antigen recognition. Mechanistically, primary memory CD8+ T cells expressed higher levels of IL-2Rβ (CD122) than did tertiary memory CD8+ T cells and were more sensitive to IL-15 stimulation, resulting in differential activation of the transcription factor STAT5. Activation of this signaling pathway triggered primary memory CD8+ T cells to synthesize core 2 O-glycans on selectin ligands, traffic specifically to inflamed tissues, and provide protection against viral infection. Thus, this study demonstrates that diverse memory CD8+ T cell populations exhibit different capacities to generate core 2 O-glycans and subsequently traffic to inflamed tissues following acute infection or injury, a finding with broad implications for vaccine design and immunotherapeutic strategies.

Presenter: Ogawa, Hiromi

T cell abnormalities in Good Syndrome: not just a B cell deficiency

Hiromi Ogawa, Forum Patel, Michiko Shimoda, Emanual Maverakis

UC DAVIS School of Medicine

Good syndrome is a rare immunologic disease characterized by hypogammaglobulinemia in the setting of thymoma. It was first described by Dr. Robert Good in 1954. Clinical studies have demonstrated that Good syndrome is associated with hematological disorders, which include anemia in over 50% patients, a low white blood cell count, thrombocytopenia and neutropenia. The immunological studies reveal low to absent B cells in the peripheral blood and an inverted CD4/CD8 T cell ratio. Patients with Good syndrome often experience pathogenic infections as well as other presentations common with other B cell deficiencies such as X linked agammaglobulinaemia (XLA) and common variable immune deficiency (CVID). For example, patients with Good syndrome are susceptible to opportunistic infections and other disorders that are linked to cell-mediated immunity, which indicates that these patients have dysregulation in both their humoral and cellular immunity. In this study, we examined the immune profile of PBMCs in Good syndrome patients by flow cytometry and their cytokine secretion profiles following stimulation with anti-CD3/28 and/or rIL-2. Our results demonstrate that Good syndrome CD4 T cells express higher levels of HLA-DR compared to healthy controls. In addition, Good syndrome patients had significantly elevated Th2 type cytokine production following anti-CD3/28 stimulation. On the other hand, Th1 type cytokine production was significantly reduced in PBMCs following stimulation with rIL-2 and/or with anti-CD3/28. These findings indicate that, in addition to their lack of a humoral response, Good syndrome patients also have imbalanced cytokine production by T cells, which may be one reason why they are predisposed to infections linked to cell-mediated immunity. Developing a better understanding of the mechanism of immune dysregulation might be helpful for treating patients with Good syndrome.

CD28-CD80 Interactions Control Regulatory T Cell Motility and Immunological Synapse Formation

David C Parker, Timothy J. Thauland, Yoshinobu Koguchi, Michael L. Dustin

Oregon Health & Science University

Regulatory T cells (Tregs) are essential for tolerance to self and environmental Ags, acting in part by downmodulating costimulatory molecules on the surface of dendritic cells (DCs) and altering naive CD4 T cell–DC interactions. In this study, we show that Tregs form stable conjugates with DCs before, but not after, they decrease surface expression of the costimulatory molecule CD80 on the DCs. We use supported planar bilayers to show that Tregs dramatically slow down but maintain a highly polarized and motile phenotype after recognizing Ag in the absence of costimulation. These motile cells are characterized by distinct accumulations of LFA-1–ICAM-1 in the lamella and TCR-MHC in the uropod, consistent with a motile immunological synapse or "kinapse." However, in the presence of high, but not low, concentrations of CD80, Tregs form stationary, symmetrical synapses. Using blocking Abs, we show that, whereas CTLA-4 is required for CD80 downmodulation, CD28–CD80 interactions are critical for modulating Treg motility in the presence of Ag. Taken together, these results support the hypothesis that Tregs are tuned to alter their motility depending on costimulatory signals. The Journal of Immunology, 2014, 193: 5894–5903.

Dissecting the role of the CD4 transmembrane domain in T cell activation

Heather Parrish, Madeline Keenen, Neha Deshpande, Michael Kuhns

University of Arizona

Not to be placed on the website

Differential ASC Requirements Reveal a Key Role for Neutrophils to the Acute IL-1β Response to Pseudomonas aeruginosa

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Infection of macrophages and dendritic cells with Pseudomonas aeruginosa infection leads to the release of the potent pro-inflammatory cytokine IL-1β through the activation of the NLRC4 inflammasome complex. Previous genetic studies have shown that the molecular components of this complex, including NLRC4, ASC and Caspase-1, are required for the IL-1β response by macrophages and dendritic cells to P. aeruginosa infection. Surprisingly, ASC-/- mice were not deficient in mounting an IL-1β response in vivo to an acute P. aeruginosa infection. Upon further investigation of this discrepancy of the ASC requirement for IL-1β release in vitro but not in vivo, we identified that neutrophils are the predominant cell type that is initially recruited upon acute peritoneal or pulmonary P. aeruginosa infection and, concomitantly, these cells upregulate pro- IL-1β expression and release the mature IL-1β cytokine. Importantly, depletion of neutrophils in vivo leads to significantly lower IL-1β release in ASC-/- mice as compared to WT mice. These results were validated with purified murine and human neutrophils, which secreted IL-1β in response to P. aeruginosa. In corroboration of the in vivo phenotype, the murine neutrophil IL-1β response was predominantly ASC-independent. Interestingly, there appear to be multiple levels of regulation of the NLRC4 inflammasome complex in neutrophils, since Pseudomonas and Salmonella typhimurium, both of which are bacterial pathogens that trigger the NLRC4 inflammasome, have differential requirements of ASC, NLRC4 and Caspase-1 for IL-1β release. Our findings identify murine and human neutrophils as a significant and important cellular source of in vivo IL-1β production in response to P. aeruginosa, and these findings reconcile the observed discrepancy between previous in vitro experiments with macrophages and dendritic cells and the observed in vivo phenotype.

Presenter: Pestal, Kathleen

The role of ADAR1 in nucleic acid sensing

Kathleen Pestal, Dan Stetson

University of Washington

Adenosine deaminases that act on dsRNAs (ADARs) deaminate adenosines in pre-mRNA, noncoding RNAs and viral RNAs to create inosines. Inosine can alter codons and splice forms, 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. Recently, mutations in ADAR1 have been shown to cause Aicardi-Goutieres Syndrome (AGS), a genetically determined inflammatory disorder of the brain and skin. Previously identified AGS mutations occur in several enzymes that act on a common pathway of retroelement metabolism, where loss of their individual functions results in an overstimulation of innate DNA sensing. However, the mechanism by which the loss of ADAR1 results in an upregulation of Type I interferon signaling is currently unknown. Mice that are deficient in ADAR1 do not survive past embryonic day 12 due, in part, to a failure of the hematopoietic system. Interestingly, when we crossed Adar-/- mice to MAVS-deficient mice, we rescued the interferon (IFN) signature in embryos. Furthermore, we have now generated the first live Adar-/- mice, as the IFN signature is only partially responsible for embryonic lethality. The rescue of the IFN signature and the live births of Adar/Mavs DKO mice enabled the first analysis of the role of ADAR1 in development independent from the pathological IFN response. Together, our preliminary findings reveal a novel disease mechanism that may contribute to AGS caused by ADAR mutations: dysregulation of the MAVS-dependent antiviral response. Moreover, we show that ADAR1 is required for the normal development of kidneys, gastrointestinal tract, and B cells, revealing a function in development that is distinguishable from ADAR1 regulation of the MAVS pathway. Based on these data, we hypothesize that ADAR1 has two independent functions: regulation of the MAVS-dependent antiviral response, and control of organ development. We further hypothesize that AGS is caused by defects in the first function of ADAR1, not the second.

The Effect of the VHL/HIF Pathway on CTL Memory Differentiation and Function

Anthony Phan, Andrew L. Doedens, Ananda W. Goldrath

University of California, San Diego

During response to infection, T cells become activated in lymphoid tissues and traffic to a myriad of host tissues in order to perform effector functions. Upon entering peripheral tissues, environmental signals, such as cytokines and nutrient availability, likely instruct responding T cells thereby tailoring effector responses. CD8+ cytotoxic T lymphocytes (CTL) are essential for the clearance of intracellular pathogens, such as viruses, and likely utilize environmental signals to modulate their cytolytic function. We have found that the von-Hippel-Lindau/Hypoxia Inducible Factor (VHL/HIF) pathway, the central transcriptional response to hypoxia, is a critical regulator of CTL differentiation and function. Strikingly, in response to acute infection, conditional deletion of VHL in CD8+ T cells results in CTL that expand poorly and fail to upregulate KLRG1, a marker of shorter-lived CTL, in comparison to wildtype CTL. Despite poor expansion and inability to form this shorter-lived CTL population, VHL-deficient CTL appear to form populations of long-lived memory cells that respond to secondary challenge despite alterations in expression of many transcription factors involved in effector and memory differentiation and perturbation of CD8+ T cell metabolism. These effects are likely due to alterations in expression of HIF targets as well as indirect effects due to HIF-dependent metabolic changes. We find that constitutive HIF activity due to VHL deletion in CD8+ T cells has profound effects on differentiation, maintenance, and function of long-lived memory CD8+ T cells following acute infection impacting protective long-term immunity.

Interleukin-1 signaling drives hematopoietic stem cells towards precocious myeloid differentiation at the expense of self-renewal

Eric Pietras, Cristina Mirantes-Barbeito, Sarah Fong, Dirk Loffler*, Ranjani Lakshminarasimhan, Chih Peng Chin, Jose-Marc Techner, Britta Will°, Claus Nerlov#, Ulrich Steidl°, Timm Schroeder*, and Emmanuelle Passegue

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Chronic inflammatory diseases such as gout, rheumatoid arthritis and type II diabetes are debilitating conditions driven by aberrant immune system activation. Importantly, these diseases are characterized by deregulated production of blood cells leading to severe hematological complications, including anemia and overproduction of myeloid cells, which contribute to disease pathology. Although increased levels of the pro-inflammatory cytokine interleukin-1 (IL-1) is a key driver of these diseases, its role in regulating their hematological features is largely unknown. Here, we demonstrate that IL-1 directly impacts the fate of rare hematopoietic stem cells (HSCs) that normally provide lifelong maintenance of all blood cell lineages. Using continuous single-cell tracking technology and molecular analyses, we show that IL-1 accelerates HSC cell division and directly instructs HSC differentiation along the myeloid lineage via rapid, precocious activation of the myeloid transcription factor, PU.1. We find that this mechanism primes HSCs to rapidly produce myeloid cells in response to acute need following myeloablative chemotherapy. Importantly however, chronic exposure to IL-1 severely restricts HSC lineage output, leading to continued myeloid overproduction at the expense of the lymphoid and erythroid lineages. Moreover, IL-1 severely erodes the self-renewal and regenerative capacity of HSCs, resulting in their failure under replicative challenges such as serial transplantation. Thus, our results identify a critical regulatory circuit by which IL-1 directly regulates myeloid differentiation in HSCs, and demonstrate that IL-1 functions as a double-edged sword, accelerating myeloid production in response to acute needs while disrupting HSC self-renewal and lineage output in the context of chronic exposure. We are now identifying the contribution of this circuit to deregulated blood production in several models of IL-1-driven chronic inflammatory disease, investigating the mechanisms by which IL-1 signaling activates PU.1, and further characterizing the changes in HSC fitness caused by chronic IL-1 exposure. Collectively, these findings provide critical insight into the effects of IL-1 on HSC function and blood homeostasis. They also provide a basis for understanding and targeting deregulated blood production that contributes to the pathogenesis of a wide array of IL-1-driven chronic inflammatory diseases.

Characterizing the metabolic interaction of Legionella pneumophila with its protozoan and metazoan hosts

Jordan Price, Russell Vance

University of California, Berkeley

The intersection of infection and host cell metabolism is an underexplored realm of host-pathogen interaction. For many intracellular bacterial pathogens, is unclear how the metabolic environment within host cells affects bacterial survival, replication, and pathogenicity. The mammalian macrophage displays a high degree of metabolic plasticity, and is the preferred host cell for many species of pathogenic intracellular bacteria. The capacity of macrophages to remodel their metabolism in response to diverse stimuli may be one reason why these cells are routinely exploited by enterprising intracellular pathogens as a replicative niche. The facultative Gram-negative intracellular bacteria Legionella pneumophila is particularly well-suited for studying the interaction between host cell metabolism and bacterial pathogenesis, as it can infect a wide range of host cells by targeting conserved cellular processes, including host metabolism. While it naturally infects free-living amoebae, Legionella can also infect human alveolar macrophages and cause the respiratory illness known as Legionnaire's disease. Many previous studies of Legionella infection have focused on how the bacteria manipulate host trafficking machinery to establish a replicative niche within its host cell, the so-called Legionella-containing vacuole. Less attention has been given to the metabolic state of the Legionella-infected macrophage, the nutrients Legionella consumes during infection, and how the mammalian immune response has evolved to respond to pathogenic metabolic perturbation. Here we present our preliminary findings describing the metabolic state of Legionella-infected macrophages in vitro, as well as data suggesting that perturbation of macrophage metabolism during infection has a dramatic impact on the ability of Legionella to replicate intracellularly. These studies expand our understanding of the dynamic metabolic interchange between host and intracellular pathogen during infection.

NAIP proteins are essential for inflammasome formation in response to cytosolic flagellin and type III secretion system components

Isabella Rauch, Kang Chulho, Vance Russel E.

University of California, Berkeley

Inflammasomes are a family of multiprotein cytosolic complexes that activate inflammatory caspases and host defense upon pathogen detection. The NLRC4 inflammasome can be activated by cytosolic presence of flagellin, or by the type III secretion system (T3SS) rod or needle proteins of bacteria. Recognition of these pathogen ligands is mediated by the NAIP family of NLRs, with NLRC4 serving as an adaptor to recruit Caspase 1 downstream of the NAIPs. Due to the highly homologous nature of the mouse NAIP genes arranged in one cluster at chromosome 13, targeting individual NAIPs for deletion with conventional methods has only been successful for NAIP5. Due to the redundancy of NAIP5 with NAIP6 in binding flagellin, Naip5-/- animals are only partially defective for responses to flagellin. The in vivo role of individual NAIPs is still unclear. For further clarification of the role of NAIPs in vivo, specific knockout strains are essential. The newly developed CRISPR/Cas9 method of genome editing allows to specifically target small regions in the genome, making it the ideal method to create such knockouts. We designed a guide RNA targeting all mouse NAIPs, thereby creating several different knockout lines with one targeting. We successfully created Naip1-/-, Naip2-/- and Naip 1-6 (cluster) -/- mouse lines. Infection of Naip1-/-, Naip2-/-, Naip5-/- and Naip1-6 -/- macrophages with Salmonella typhimurium shows that no single NAIP is required for pyroptosis upon Salmonella infection. However, loss of NAIP1-6 phenocopies NLRC4 deficiency. In vivo treatment of NAIP1-6 deficient animals with FlaTox (intracellular delivered flagellin) shows a complete loss of reaction to this toxin comparable to NLRC4 deficient animals, proving that NAIPs are essential for intracellular flagellin recognition. Further analysis of these mouse lines will give valuable insight into the role of individual NAIPs in pathogen recognition.

Presenter: Resop, Rachel

HIV-1 infection increases Sphingosine-1-phosphate receptor 1 expression in the human thymus

Rachel Resop, Marc Douaisi, Josh Craft, Christel Uittenbogaart

University California, Los Angeles

Lack of T cell regeneration in HIV infected individuals 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 limited data available to elucidate the impact of HIV on these processes. We studied the effect of HIV on the receptors to Sphingosine-1-phosphate (S1P), a chemotactic sphingolipid. We have shown that human thymocytes migrate to S1P and that FTY720 inhibits migration by functioning as an agonist to S1P, thereby downregulating S1P receptor 1 (S1P-R1), the main S1P receptor responsible for response to S1P in mice and humans. 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 demonstrated that thymocytes expressing S1P-R1 respond to S1P exposure in vitro with increased Akt phosphorylation, internalization of the receptor upon binding, and downregulation of S1P-R1 mRNA. In our current work we examined the dynamics of S1P-R1 expression in the human thymus during HIV infection, which has thus far not been described. Two series of NSG mice implanted with human fetal thymus/liver (thy/liv) grafts and infected with CXCR4- or CCR5-tropic HIV-1 were used to analyze the effect of HIV on S1P-R1 in the human thy/liv implant. Persistent infection and immune activation were verified by demonstrating that two Interferon-alpha secondary genes, MxA and ISG15, were 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 thymocytes at two time points post HIV infection. Hence, S1P-R1 function after HIV infection of the human thy/liv 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. The mechanism of the increase in S1P-R1 and KLF2 was investigated and our results show that Tumor Necrosis Factor alpha (TNF-A), Interferon-alpha and Interferon gamma were elevated in the infected thy/liv implant. Upon treatment with exogenous TNF-A, S1P-R1 expression level, as measured by the Mean Fluorescence Intensity, was increased to a statistically significant extent in mature thymocytes, indicating that secretion of this cytokine in the thymus may contribute to the increase in S1P-R1 expression. 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 minor subset expressed S1P-R1 and likely migrated to the periphery, indicating that entry into the thymus and development are likely functional during early infection. 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: Roan, Florence

Characterization of phenotypically and functionally heterogeneous ILC1 subsets in human peripheral blood

Florence Roan, Thomas A. Stoklasek, Steven F. Ziegler

Benaroya Research Institute

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CXCL12-expressing reticular cells are a novel stromal cell type in the germinal center dark zone

Lauren B. Rodda, Oliver Bannard and Jason G. Cyster

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Germinal centers (GCs) are tightly organized into a light zone (LZ) and a dark zone (DZ). GC B cells respond to CXCL12 to access the DZ niche and undergo efficient mutation and selection, but the source of this critical chemokine is unknown. Using CXCL12-gfp reporter mice, we identified a new subset of stromal cells we termed CXCL12-expressing reticular cells (CRCs) in the GC DZ and T zone proximal region of the primary follicle. These cells form tight, irregular nets in spleen and peripheral lymph node GCs in response to LCMV infection, flu infection and SRBC immunization. CRCs are also present in chronic GCs in mesenteric lymph nodes and Peyer's patches. Distinct from follicular dendritic cells (FDCs) and fibroblastic reticular cells (FRCs), CRCs are low or negative for CD35, CD16/32, Mfge8, FDCM2, ER-TR7 and Type IV collagen. Unlike FDCs, CRCs are not dependent on LTa1β2 and TNF signaling for short-term maintenance. CXCR4 signaling was required for CRC distribution through the DZ, likely reflecting a dependence on GC B cell proximity for their structural maintenance. Our findings identify CRCs as a major stromal cell type in the GC DZ and suggest they support critical activities of GC B cells in the DZ niche.

Differential regulation of lncRNAs during effector and memory CTL development

Walter Rogal, Nicholas Bild, Gustavo Martinez, Matthew Pipkin

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The mammalian genome encodes thousands of long non-coding RNAs (lncRNAs). However, the functions in cellular differentiation of most lncRNAs is unclear, and their role(s) in the development of effector and memory cytotoxic T lymphocytes (CTL) from naive CD8 T cells is essentially unexplored. Using several RNA-seq approaches to analyze effector and memory CTL development in a cell culture system that is modulated by IL-2 receptor stimulation in vitro, and in bona fide memory precursor effector cells (KLRG1- CD127+) and short-lived effector cells (KLRG1+ CD127-) responding to lymphocytic choriomeningitis virus (LCMV) infection in vivo, we have identified thousands of differentially expressed lncRNAs that might contribute to CTL differentiation. A substantial fraction of differentially expressed lncRNAs from ex vivo effector and memory CTL were regulated by the strength of IL-2 receptor stimulation in vitro, and many were controlled transcriptionally by these signals. Our data is consistent with the possibility that lncRNAs promote CTL differentiation. We are currently applying retroviral-mediated transgene expression and RNAi approaches to decipher the function(s) of differentially expressed lncRNAs during CD8 T cell differentiation.

A wave of regulatory T cells into neonatal skin mediates tolerance to commensal microbes

Tiffany C. Scharschmidt, Kimberly S. Vasquez, Hong-An Truong, Sofia V. Gearty, Mariela L. Pauli, Audrey Nosbaum, Iris K. Gratz, Michael Otto, James J. Moon, Jan Liese, Abul K. Abbas, Michael A. Fischbach, Michael D. Rosenblum

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Each centimeter of our skin contains over a million lymphocytes and a million commensal bacteria. Very little is known about how the cutaneous immune system continuously senses antigens from commensal bacteria without causing destructive inflammation. To elucidate how adaptive immune tolerance is established and maintained to skin commensal microbes, we engineered a prototypical skin commensal (S. epidermidis) to express the model antigen 2W (Epi-2W), allowing us to comprehensively analyze commensal-specific CD4+ T cell responses in the context of both a polyclonal T cell repertoire and a complex microbiome. Using this system, we observed that skin colonization during a defined period of neonatal life was required to establish tolerance to Epi-2W. This window of time is characterized by an abrupt wave of highly activated regulatory T cells (Tregs) that accumulate in skin during the first weeks of life. Inhibition of Treg migration into skin during this period completely abrogated tolerance to Epi-2W. Our results demonstrate that the host-commensal relationship in skin relies on a unique population of Tregs that control antigen-specific responses directed at skin microbes, and that there is a critical developmental window in which this tolerance is established. This suggests that composition of the cutaneous microbiome in the neonatal period has formative effects that shape the adaptive immune response to bacterial antigens and that disrupting the cutaneous host-commensal relationship early in life may have enduring health implications.

SUPPRESSION OF SYSTEMIC AUTOIMMUNITY BY THE INNATE IMMUNE ADAPTOR STING

***Shruti Sharma, Allison M. Campbell*, Jennie Chan*, Stefan A. Schattgen*, Gregory M. Orlowski*, Ribhu Nayar, Annie H. Huyler, Kerstin Nundel, Chandra Mohan, Leslie J. Berg, Mark J. Shlomchik, Ann Marshak-Rothstein† and Katherine A. Fitzgerald* † (* and † contributed equally)**

University of Massachusetts Medical School

Cytosolic DNA-sensing pathways that signal via STING mediate immunity to pathogens and also promote autoimmune pathology in DNAseII/III (Trex1)-deficient mice. In contrast, we report here that STING potently suppresses inflammation in a model of systemic lupus erythematosus (SLE). Lymphoid hypertrophy, autoantibody production, serum cytokine levels, and other indicators of immune activation were markedly increased in STING-deficient autoimmune-prone mice compared to STING-sufficient littermates. As a result, STING-deficient autoimmune-prone mice had significantly shorter lifespans than controls. Importantly, TLR-dependent systemic inflammation during TMPD (pristane)-mediated peritonitis was similarly aggravated in STING-deficient mice. Mechanistically, STING-deficient macrophages failed to express negative regulators of immune activation, including IDO-1, and thus were hyper-responsive to TLR ligands, producing abnormally high levels of pro-inflammatory cytokines. This hyper-reactivity corresponds to dramatically elevated numbers of inflammatory macrophages and granulocytes in vivo. Collectively these findings reveal an unexpected negative regulatory role for STING, having important implications for STING-directed therapies.

Presenter: Shaw, Laura

The role of Id proteins in CD4+ T cell memory

Laura Shaw, Kyla D Omilusik and Ananda W Goldrath

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When activated by antigen in the context of MHC class II, CD4+ T cells initiate a specific transcriptional program that affords them the unique ability to differentiate into multiple helper (Th) populations, such as Tfh and Th1. Tfh cells are essential for germinal center responses and supporting long-lived antibody production, whereas Th1 cells are responsible for defending against intracellular pathogens. As the immune response wanes, the majority of the antigen-specific T cells will die but a few CD4+ T cells will survive indefinitely to establish memory populations, providing long-lived protection against reinfection. This ability of lymphocytes to 'remember' is the basis for protection following vaccination. Analogous to the CD8+ T cell response, we propose that E protein transcription factors and their natural inhibitors, Id proteins, play a major role in coordinating effector and memory CD4+ T cell differentiation. Using mice expressing knocked-in fluorescent reporters, we have identified specific expression of Id2 and Id3 in effector (Th1) and memory (Tfh) populations, respectively, following infection. Furthermore, with conditional knock-out models, we demonstrate an essential role for Id2 and Id3 in promoting the generation and survival of Tfh and Th1 populations. Understanding the transcriptional network that leads to the development and localization of these CD4+ T cell subsets will prove useful in designing vaccines that elicit an optimal cellular immune response.

Presenter: Sheih, Alyssa

Airway epithelial cells promote allergic airway inflammation to cockroach allergen through production of GM-CSF in a MyD88-dependent manner

Alyssa Sheih, Steven F. Ziegler

University of Washington

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Presenter: Shimoda, Michiko

A mouse model for chronic inflammatory diseases spontaneously develops CD4/CD8 double-positive T cell leukemia/lymphoma

Michiko Shimoda, Pandelakis A. Koni, Mingqiang Ren, Takeshi Tsubata, Samir N. Khleif, Emanual Maverakis

University of California, Davis

Not to be placed on the website

Control of T cell proliferation, differentiation, and effector function by the adaptor protein BCAP

Mark Singh, Shivani Srivastava, Jeffrey Duggan, Jessica Hamerman, Daniel J. Campbell

Benaroya Research Institute at Virginia Mason

One of the central signaling networks required for a robust effector T cell response is the phosphoinositide 3-kinase (PI3K) network. While much is known about the role of the PI3K pathway in the T cell response, the molecular mechanisms involved in regulating PI3K function during T cell activation are poorly understood. The adaptor protein B Cell Adaptor for PI3K (BCAP) has previously been detected in B cells and myeloid cells, and there is evidence suggesting that BCAP is involved in the regulation of PI3K signaling pathways in these cell populations. We have generated novel data demonstrating the role of BCAP in ensuring optimal T cell activation, proliferation and differentiation. We have determined that although BCAP is not expressed in naive T cells, BCAP expression is detected in effector memory T cell populations, and can be rapidly induced in naive T cells upon T cell activation in vitro. In addition, BCAP deficient T cells are hypo-proliferative, and have attenuated signaling cascades downstream of PI3K in vitro. To study the role of BCAP in T cells in vivo, we infected wild type and BCAP deficient mice with the pathogen Lymphocytic Choriomeningitis Virus (LCMV). At 7 days post infection, notable skewing in the effector CD8+ T cell populations was observed, with an increased frequency of KLRG1loCD127hi memory precursor effector cells (MPECs) at the expense of KLRG1hiCD127lo short lived effector cells (SLECs). In addition, use of mixed bone marrow chimeras showed that wild type T cells have a significant competitive advantage over BCAP deficient T cells in vivo. Collectively, these data suggest that BCAP may have a non-redundant role in determining T cell fate in vivo.

Presenter: Slichter, Chloe

Inflammatory signals license human MAIT cell effector function

Chloe Slichter, Andrew McDavid, Hannah W. Miller, Greg Finak, Brenda J. Seymour, John P. McNevin, Gabriela Diaz, Julie L. Czartoski, M. Juliana McElrath, Raphael Gottardo, and Martin Prlic

University of Washington

Not to be placed on the website

Presenter: Song, Jing

Manipulation of lymphocytes by sphingolipid analog AD-2900 for therapeutic purposes

JING SONG, O. Almogi-Hazan, S. Gatt , A. Dagan, R. Or

The Hebrew University of Jerusalem

Sphingolipids have significant roles in the immune system. Sphingolipid and its derivatives are involved in common signalling pathways which control the main stages of immune cells development and function. The use of sphingolipid analogues, such as FTY-720, is currently under investigation as a therapy for different immune disorders. We hypothesize different sphingolipid analogues have different effect on immune cell function and therefore different analogues can be used for intervention in various diseases. A group of sphingolipid analogues have been synthesized in our laboratory. Based on their effect on lymphocyte cell proliferation, we have selected one of our synthesized sphingolipid analogues, AD-2900. In this research we have characterized the properties of AD-2900 and examined its influence on lymphocytes function. We found that AD-2900 is an antagonist for all the S1P receptors 1 to 5; Specially, AD-2900 shows high antagonism property for S1P5 (IC50 = 0.405μM). Moreover, AD-2900 treatment leads to a significant but reversible down-regulation of S1PR1 cell surface expression on hPBLs. However, AD-2900 is not phosphorylated either by SphK1 or SphK2. We have tested the effect of AD-2900 on immune activation. In hPBL, AD-2900 inhibited T cell proliferation and induced apoptosis dose-dependently. However, it had a unique effect on immune activation comparing to FTY-720 and S1P. The inhibitory effect of the analogue on hPBL proliferation was dependent on cAMP reduction and calcium signal transduction, but not on phospholipase C (PLC) activation (which participates in S1P's effect). The effect of FTY-720 on hPBL proliferation was not associated with any of these pathways. In addition AD-2900 induced ERK phosphorylation in non-activated cells. However, the Phosphorylation patterns were different as compare to cells treated with the other analogues. In order to investigate whether AD-2900 plays a role in lymphocyte localization in vivo, C57BL/6 female mice were oral administrated AD-2900 in their drinking water in different dosages; FTY-720 serves as a positive control. The results show that, in contrast to FTY-720, AD-2900 had no impact on blood WBC counting after 48h administration. However, while FTY-720 treatment decreased spleenocyte counting, AD-2900 significantly increased the number of WBC in the spleen. Modulations in the surface expression levels of S1PR1 and the chemokine receptor CCR7 on lymphocytes are involved in the circulation of these cells between the lymphatic organs and the blood. We found that AD-2900 significantly down-regulates the surface expression S1PR1 on lymph node and blood WBC. The expression of CCR7 on mice spleen cells was significantly up-regulated by AD-2900. Notably, FTY-720 had no influence on CCR7 expression in the spleen. In blood cells, both AD-2900 and FTY-720 treatments caused significant down-regulation of CCR7. Currently, we are expanding our knowledge regarding the specific sub-populations of cells which are affected by AD-2900. Hopefully, the results of this study may allow us to use our innovative sphingolipid analogue AD-2900 to develop an effective immune-modulatory drug for immunotherapy of immune related disorders.

Identifying new human pathways involved in bacterial toxin resistance using a novel forward genetic screen

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Bacterial toxins are essentials virulence factors allowing bacterial entry, persistence, and dissemination. Toxins are key players in host-pathogen interaction; therefore identifying the mechanisms of cellular resistance will be of fundamental importance to fight against numerous human pathogens. Furthermore, because of their highly specific action in cells, through their co-evolution with host, bacterial toxins are also selective and efficient tools to characterize cell biological pathways. For some toxins, even low amounts of toxin are sufficient to recapitulate the deadly symptoms of the infection. As part of our host defense response, cells are able to counteract toxins effects by sensing them or their effects and inducing biological responses and repair. However these mechanisms of cell resistance remain unknown. To answer this intrinsic question, we undertaken a forward genetic screening approach using transposon mutagenesis as an unbiased in vitro strategy to understand the mechanisms of cells resistance to toxins and the mechanisms of cellular repair. Specifically, this strategy relies on piggyBac transposon mutagenesis to generate a library of mutagenized cells presenting gain and lost-of function profile. The selection of resistant mutant clones and NextGen sequencing let us identify transposon insertions sites and candidate genes that contribute to protection. By combining the analyses of toxins sharing the same entry, the same trafficking or the same cellular target, we were able to identify candidate resistance genes that counteract multiple steps of bacterial toxin pathogenesis. We were also able to identify resistance genes specific to each toxin.

Turn Down For What: Loss of CD4 expression Facilitates Antigen Exchange Between 33D1+ Dendritic Cells

J. Michael Stolley, Daniel J. Campbell

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MHC class-restriction, the concept that helper T cells expressing the co-receptor CD4 encounter antigen in the context of MHC-class II while cytotoxic T cells expressing the co-receptor CD8 receive TCR activation through MHC-class I, is immunological dogma. Paradoxically, dendritic cells (DCs) which express high levels of MHC molecules differentially express the T cell co-receptors CD4 and CD8a, and these are in-fact used to subset distinct populations of DCs within secondary lymphoid tissues. Why DCs concurrently express MHC molecules and T cell co-receptors has puzzled immunologists since the discovery that DCs expressed CD4 or CD8a in the 1990â€™s. However, studying the function of these molecules on DCs is confounded by the fact that their T cell counterparts are absent in CD4 or CD8a-deficient mice. Here we provide a glimpse into the potential function of CD4 on DCs. CD4 expression on 33D1+ DCs is markedly diminished following TLR agonist exposure in vivo in a cell-intrinsic manner. Using chimeric mice in which CD4-deficiency can be restricted within the DC compartment, we provide evidence suggesting loss of CD4 expression on 33D1+ DCs facilitates the exchange of antigens between 33D1+ DCs. Consistent with these findings, peptide-loaded CD4-deficient DCs relinquished more antigen to congenically marked DCs in vitro than CD4-sufficient DCs. Therefore, we suggest a model by which CD4 down-regulation succeeding phagocytosis (and subsequent TLR activation) of pathogenic material expedites the exchange of microbial peptides from activated to non-activated DCs; rapidly increasing the overall number of DCs which can initiate CD4+ T cells responses.

Identification of a suppressor of autoimmune disease that affects deoxynucleotide synthesis in T cells

Gautam Sule, Tanya Tarasenko, Wenjie Zheng, Bethany Scott and Silvia Bolland

NIAID, NIH

FcγRIIB-deficient mice represent a well-characterized animal model of systemic lupus erythematosus. They develop spontaneous anti-nuclear antibodies (ANA) and fatal glomerulonephritis when on the C57BL/6 (B6) background (B6.FcγRIIB -/- mice). In contrast, the same mutation on the BALB/c background (BALB.FcγRIIB -/- mice) is phenotypically benign, indicating differences in lupus susceptibility between the BALB/c and B6 strains. After extensive backcrossing, we narrowed down the genomic interval to a 151KB genomic fragment on chromosome 12 responsible for the suppressive/protective effect in BALB/c mice. We generated a transgenic mouse line expressing this BALB/c genomic region directly in the C57BL/6 background (A12 Tg) and subsequently crossed to B6.FcγRIIB -/- mice to test the putative protective effect. The BALB/c-derived A12 transgene was able to suppress the spontaneous disease that normally develops in B6.FcγRIIB -/- mice: it reduced spontaneous germinal center formation, serum autoantibody titers and proteinuria. A12 transgene also reduced the number of activated and memory T cells, as well as the number of follicular helper T cells when compared to the B6.FcγRIIB -/- mice. Additional studies suggest that reduction in the autoimmune phenotype is T cell-intrinsic. Rrm2 is the only protein-coding gene present in the A12 transgene. RRM2 forms one of the subunits for ribonucleotide reductase, which catalyzes the rate-limiting step for the production of deoxyribonucleotides from ribonucleotides. We are in process of characterizing the mechanism by which Rrm2 is able to have this protective effect. Finally we will test the ability of Rrm2 to suppress autoimmunity in other susceptible strains/lupus models to identify the translatability of RRM2 as a target for therapy.

What does the fox say? Regulation of Foxo1 in T cell homeostasis

Jenna M. Sullivan, Kerri Thomas and Daniel J. Campbell

University of Washington

The Foxo (forkhead box O) family of transcription factors is important for maintaining homeostasis of both effector and regulatory T cells (TR). Interestingly, Foxo1 is necessary for proper development and function of TR and absence of Foxo1 results in fatal inflammation due to TR dysfunction. During T cell activation, phosphorylation of Foxo1 by Akt results in inactivation its and translocation from the nucleus. We have bred mice that contain a floxed non-phosphorylatable Foxo1 allele under control of CD4-cre (CD4cre-Foxo1CA), resulting in constitutive activation of Foxo1 in both CD4 and CD8 T cells. Mice which are unable to properly regulate Foxo1 have reduced body weight, chronic inflammation and develop spontaneous autoimmunity. CD4cre-Foxo1CA displaying symptoms of autoimmunity have tissue damage in the lung along with increases in eosinophils, alveolar macrophages and mucus production. In the periphery CD4cre-Foxo1CA mice have reduced TR number, increased activated effector T cells and an altered B cell compartment. Together this data demonstrates that proper regulation of Foxo1 is essential for T cell homeostasis and prevention of autoimmunity.

Presenter: Sun, Wenxiang

Increased expression of TLR3 in a transgenic model uncovers differential competition among endocytic TLRs

Wenxiang Sun, Wenjie Zheng, Silvia Bolland

NIH

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Calcium Signaling in T helper cell Primary and Secondary Responses

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CD4+ helper T cells play a critical role in the immune response to infectious disease as they interact with macrophages and dendritic cells and activate B cells and cytotoxic CD8+ T cells. CD4+ T cells are essential for the pathogen specific responses of B cells and CD8+ T cells and are essential for the proliferation, differentiation, and memory cell formation of CD8+ T cells. Since helper T cells have such a central role in the adaptive response many studies have examined how they are activated and calcium (Ca2+) signaling is an important way to measure activation. Calcium is a universal second messenger and plays an important role in the regulation of cell differentiation, gene transcription and other effector functions of the cell. Calcium signaling has been well characterized in lymphocytes and the characteristics of the calcium signal for specific helper T cell subsets such as TH1, TH2 and TH17 have been identified. However, the unique calcium signal of helper T cells involved in primary and secondary responses is not yet well elucidated. To address this question, we are using two helper T cells from TCR transgenic mice specific for Listeriolysin O (LLO) of Listeria monocytogenes called LLO118 and LLO56. These helper T cells differ by only 15 amino acids and are specific for the same Listeria monocytogenes epitope, yet they have dramatically different primary and secondary responses. After preparing and isolating our desired helper T cell populations we are using ratiometric live cell calcium imaging to measure calcium profiles of T cells after primary and secondary stimulation. This project will help to elucidate the role that calcium signaling plays in helper T cell activation in primary and secondary responses and should produce additional understanding into activation of helper T cells in response to infectious disease.

Presenter: Tenthorey, Jeannette

Towards an understanding of NAIP/NLRC4 inflammasome assembly

Jeannette Tenthorey

University of California, Berkeley

NAIP/NLRC4 inflammasomes assemble upon detection of conserved bacterial proteins in the host cell cytosol. Once assembled, inflammasomes serve as a scaffold that activates the protease CASPASE-1. Active CASPASE-1 initiates inflammation by cleaving pro-IL-1ÃŸ and IL-18 to their active forms, and by inducing a lytic form of cell death known as pyroptosis. However, the mechanism by which inflammasomes bind their ligands, assemble a signaling-competent oligomer, and coordinate CASPASE-1 activation remains elusive. Mice express multiple paralogs of NAIP that respond to distinct bacterial ligands. By generating chimeras between different NAIP paralogs, we found that ligand discrimination is not mediated by the leucine-rich-repeat (LRR) domain, as expected, but rather by several internal helical domains of NAIP. Furthermore, we found that each NAIP protomer must bind its cognate ligand to assemble into inflammasomes. To further refine the ligand binding site on NAIPs and to elucidate the mechanism by which ligand binding activates NAIPs, we have initiated structural studies of the NAIP/NLRC4 inflammasome. We expect these structural studies will be critical in clarifying how inflammasome assembly is initiated in response to cytosol-accessing bacterial pathogens.

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 type-2 inflammation. 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). In a lung tissue specific IL-25 over-expression mouse model, we found that turning on IL-25 expression results in dramatic increases IL-25 protein in lung and bronchial alveolar lavage (BAL) fluid, eosinophilia, increased mucus production, and increased serum IgE levels. When WT mice are given intranasal injections of IL-25 every other day for two weeks, we found that IL-25 alone is capable of inducing Type-2 inflammation, and this response is enhanced in the presence of antigen. When an a-CD4 antibody is used to deplete CD4+ cells the Type-2 response is diminished but not completely abolished. These results suggest IL-25 is capable of activating both innate and adaptive cell types. We are currently crossing a conditional knockout in which the IL-25 receptor subunit specific to IL-25, IL-17RB, is flanked with LoxP sites to CD4- and Lysm-CRE mice. This will allow us to eliminate IL-25 signaling within specific cell types to more precisely determine which cells are responding to IL-25 and generating a TH2 response. Another important question we would like to answer is what the interdependence is between the three epithelial cytokines; IL-25, IL-33, and TSLP. Using the same model of intranasal injections every other day for two weeks, we found that the Type-2 response was severely diminished in both TSLP and IL-33 receptor knock-out mice. These results are also seen when using blocking antibodies against the TSLP or IL-33 receptors. Interestingly, the abrogated response was only observed when IL-25 was given alone. When IL-25 is administered along with Ova, the response was similar to WT. This indicates there is cross-talk between these cytokines and we are currently investigating the mechanism behind this response.

Presenter: Tinoco, Roberto

PSGL-1, an immune checkpoint regulator that promotes T cell exhaustion in chronic viral infection and cancer

Roberto Tinoco, Florent Carrette, Monique L Barraza, Jonathan Magana, Marcus W Bosenberg, Susan Swain, Linda M. Bradley

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Depletion of T regulatory cells without hampering anti-tumor immunity for improved tumor therapy

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Regulatory T (Treg) cells are one of the most important populations of immune cells with suppressor/regulatory activity. However, they also play critical role in impeding of immunosurveillance against autologous tumors. This is not so surprising as tumor cells mostly originate from normal cells of self origin thus are protected by Treg cells against anti-tumor reactions. Elimination of Treg cells from organism via administration of aCD25 mAb was repeatedly reported to be beneficial in tumor treatment. However, long-term persistence of aCD25 mAb in circulation together with its inhibitory effect on activated effector cells (CD25+) presents serious disadvantages. In order to overcome above mentioned problems, we developed a novel approach based on avidin-biotin system. We applied biotinylated aCD25 mAb (aCD25-BIO) and at selected time point, we eliminated it from the organism via administration of avidin covalently bound to N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer carrier (HPMA-avidin conjugate). Initially, we tested in vivo biological activity of aCD25-BIO in comparison to native aCD25 mAb and observed no significant differences. Its application led to decrease of Treg cells in organism and the optimal time for aCD25 mAb elimination from organism was determined to be 5 days after injection. We administered HPMA-avidin conjugate and saw steep decrease of anti-CD25-BIO within 4-8 hours afterwards and the amount of anti-CD25-BIO in sera was reduced to non detectable concentrations. Comparison of free avidin and HPMA-avidin conjugate showed that free avidin is not as effective as its conjugated counterpart. In conclusion, we assume our system is effective in terms of Treg cell depletion and subsequent anti-CD25 mAb elimination from organism, thus not hampering anti-tumor immunity.

Comparative study on efficacy of HPMA copolymer-based drug conjugates in treatment of solid and disseminated tumors

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In this study, we compared in vivo biological activity of linear (Mw ~ 27 000) and star-like (Mw ~ 250 000) N-(2-hydroxypropyl)methacrylamide (HPMA) based copolymer conjugates bearing anthracycline antibiotic doxorubicin (DOX) bound via pH-sensitive hydrazon bond. We evaluated toxicity of both conjugates and compared their anti-tumor activity in murine B-cell leukemia (BCL1) and mouse T-cell lymphoma (EL4) model. The investigation of intestinal barrier integrity via FITC-dextran assay revealed no pathology caused by neither conjugate compared to free DOX. Histological analysis, however, showed yet to be explained accumulating process in spleen positively correlating with dosage of conjugates which was also accompanied with occurrence of mitotic hepatocytes in liver parenchym. Nevertheless, no additional abnormalities were seen in other tested organs (i.e. kidney, vertebral bone marrow). In vivo anti-tumor activity showed differences in treatment of various tumors. Therapy of BCL1 leukemia by either conjugate seemed to have quite similar outcome with slightly better effects of star-like conjugate. However, it appears that long persistence of star-like conjugate could cause side toxicity. Compared to that, treatment of EL4 lymphoma seems to be more efficient when linear conjugate is used. Star-like conjugate, on the other hand, seems to promote development of anti-tumor resistance in cured mice. In conclusion, we suppose that the ideal drug-bearing conjugate should have HMW structure which could be rapidly disintegrated into small polymer chains during first few days after administration. Acknowledgement: This work was supported by Czech Science Foundation grant P301/11/0325, by the Ministry of Education, Youth and Sports grant CZ.1.07/2.3.00/20.0055 and by Institutional Research Concept RVO 61388971.

Distinct Phenotype of Pancreatic Regulatory T cells during Autoimmune Diabetes

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Regulatory T cells (Treg) are known to play a critical function in restraining inflammation in a variety of autoimmune diseases, including type 1 diabetes. However, the precise phenotype and role of Treg cells in the insulitic lesion during diabetes development remain poorly characterized. We thus sought to describe the phenotype and function of Treg cells in the pancreas in the NOD mouse over the course of insulitis, and found a striking enrichment of T-bet-dependent, CXCR3+ Treg cells in the pancreas relative to the spleen and lymph nodes. Pancreatic CXCR3+ Treg cells are induced by IFN-γ and manifest an activated phenotype, with elevated expression of a panoply of immunoregulatory molecules, and their frequency correlates negatively with the size of the insulitic infiltrate. Thus the Th1 inflammation in the insulitic lesion induces and recruits CXCR3+ Treg cells that may help to control eventual progression to overt disease.

Equine Immune Response to Corynebacterium pseudotuberculosis

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Occidental College: Department of Biology; University of California Davis: School of Veterinary Medicine

Pigeon Fever is an equine disease caused by Corynebacterium pseudotuberculosis, a gram-positive facultative anaerobic bacterium. The disease manifests in several forms: external abscesses, internal infections, and ulcerative lymphangitis. What exactly determines the manifestation a horse develops is currently unknown. Internal infection causes abscesses on the horseâ€™s organs and is associated with a mortality rate close to 40%. Internal infections are currently diagnosed by analyzing levels of antibodies to a C. pseudotuberculosis secreted toxin called phospholipase D (PLD). However, previous studies in our lab have shown that horses with external abscesses or no infection can also have high levels of antibody against PLD, showing the difficulty in diagnosing internal infections. A simpler diagnostic test for internal infections would allow for better detection and rapid antibiotic treatments, resulting in a decreased mortality rate. This project used western blot analysis to determine if recognition of specific C. pseudotuberculosis proteins could be useful as a diagnostic test. Initial results found differential recognition of C. pseudotuberculosis bacterial proteins by antibodies from horses with internal infections versus those with a single external abscess. However, strong differences were not seen between horses with multiple external abscesses and horses with internal infections in their recognition of C. pseudotuberculosis bacterial proteins.

Presenter: Vatakis, Dimitrios

Stimulant-mediated impairment of CD8 T cell immune responses

Dimitrios Vatakis, Irene Kim, Emily Lowe, Dhaval Dixit, Tim Bolan, Andrew Cross

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Cocaine has broad-ranging effects on host immunity, altering the phenotype and function of antigen presenting cells, macrophages, T cells, and the cytokines that they produce. Studies have suggested that lymphocyte exposure to stimulants results in the release of cytokines such as IL-10 and TGF-beta while others support a more pro-inflammatory profile characterized by elevated IFN-gamma and IL-6 secretion. In addition, limited studies have suggested that cocaine blunts T cell proliferative responses. Here, within the context of HIV infection, we show both in vivo and in vitro that even though stimulant exposure leads to higher activation marker expression, the effector responses of CD8 T cells are severely impaired. Thus, stimulant exposure can have major implications in human immune responses against infection and/or chronic disease.

STAT5 paralog dose governs T cell effector and regulatory function

Alejandro Villarino, Arian Laurence, Gertraud W. Robinson, Michael Bonelli, Barbara Dema, Han-Yu Shih, Hong-Wei Sun, Lothar Hennighausen, Yuka Kanno, John J. O'Shea

National Institute of Arthritis, Musculoskeletal and Skin Diseases (NIAMS), National Institutes of Health (NIH)

The transcription factor STAT5 is fundamental to the immune system. However, the relationship between its two paralogs, STAT5A and STAT5B, and the extent to which they are functionally distinct, remains controversial. We addressed this longstanding question using genetic and genomic approaches focusing on helper T cells, the key orchestrators of adaptive immunity. Our studies demonstrate that, while both control vital aspects of T cell biology, including Tfh and Treg function, STAT5B is dominant and, thus, uniquely associated with immune tolerance. DNA binding and transcriptome analyses revealed that STAT5B also dominates at the molecular level, exhibiting greater influence on both target gene selection and transcription. This disparity is largely explained by relative abundance (i.e. paralog dose) rather than primary structural variations. Collectively, our findings provide a unifying model for the unique and redundant activities of STAT5A and STAT5B, establishing that differential expression underlies functional specificity despite extensive structural homology.

Influence of myelin-specific CD8+ T cells on CD4+ T cell-initiated experimental autoimmune encephalomyelitis

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). Although the pathogenic pathways of MS are not fully understood, it is hypothesized that self-reactive T cells specific for myelin proteins initiate CNS inflammation, demyelination, and axonal damage. Myelin-specific CD4+ T cells have been the main focus of MS research but recent studies have suggested that CD8+ T cells are also involved in MS pathogenesis. We hypothesize that simultaneous recruitment of myelin-specific CD8+ T cells to the site of inflammation initiated by CD4+ T cells could influence the pathology, clinical signs, and severity of CNS autoimmune disease. To test our hypothesis, we use a mouse model of MS, experimental autoimmune encephalomyelitis (EAE) in which adoptive transfer of CD4+ T cells specific for the myelin protein myelin oligodendrocyte glycoprotein (MOG) induces CNS autoimmunity characterized by both brain and spinal cord inflammation. To determine the influence of CD8+ cells, we transfer CD8+ T cells from a TCR-transgenic mouse model expressing a TCR specific for a MHC class I-restricted epitope of myelin basic protein (MBP) into the periphery of mice just after disease is initiated by CD4+ T cells. We found that the recruitment of the MBP-specific but not control CD8+ T cells exacerbated CD4+ T cell-initiated EAE. Intriguingly, preliminary data suggest that recruitment of myelin-specific CD8+ T cells increases the number of activated CD4+ T cells, but not CD8+ T cells, within the CNS. These data suggest that the interplay between CD4+ and CD8+ T cells specific for two different myelin proteins influences the manifestation of CNS autoimmune disease.

Novel IL-2-poly(HPMA)nanoconjugate based immunotherapy

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Interleukin-2 (IL-2) possesses a strong stimulatory activity for activated T and NK cells and it is an attractive molecule for immunotherapy. Nevertheless, extremely short half-life and severe toxicities associated with high-dose IL-2 treatment are serious and limiting drawbacks. In order to increase IL-2 half-life in vivo, we covalently conjugated synthetic semitelechelic polymeric carrier based on N-(2-hydroxypropyl)methacrylamide (HPMA) to IL-2. Thus, we synthesized IL-2-poly(HPMA) conjugate containing 2-3 polymer chains per IL-2 molecule in average. Such conjugate has lower biologic activity in comparison to IL-2 in vitro. However, it exerts much higher activity than IL-2 in vivo as shown by expansion of memory CD8+ T, NK, NKT, ?dT and Treg cells. Moreover, IL-2-poly(HPMA) extremely effectively potentiates CD8+ T cell peptide-based vaccination. IL-2-poly(HPMA) shows also much longer half-time in circulation than IL-2 (~4h versus ~5min). Collectively, modification of IL-2 with poly(HPMA) chains dramatically improves its potency and pharmacologic features in vivo, which have implications for immunotherapy. To our knowledge, this is the first proof-of-concept report of the use of polymer/protein modification of IL-2 to obtain more pronounced biological activity.
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Presenter: Wirnsberger, Gerald

Jagunal-homolog 1 is a critical regulator of neutrophil function in fungal host defense

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Neutrophils are key innate immune effector cells essential to fight bacterial and fungal pathogens. Here we report that mice carrying a hematopoietic lineage-specific deletion of Jagunal homolog 1 (Jagn1) cannot mount an efficient neutrophil-dependent immune response to the human fungal pathogen Candida albicans. Global glycobiome analysis revealed marked alterations in the glycosylation of proteins involved in cell adhesion and cytotoxicity of Jagn1-deficient neutrophils. Functional analysis confirmed marked defects in neutrophil migration in response to Candida albicans infection, impaired formation of cytotoxic granules, as well as defective MPO-release and killing of Candida albicans. GM-CSF treatment protected mutant mice from increased weight loss and accelerated mortality after Candida albicans challenge. Importantly, GM-CSF also restored the defective fungicidal activity of bone marrow cells from patients with JAGN1 mutations. These data directly identify Jagn1/JAGN1 as a novel regulator of neutrophil function in microbial pathogenesis and uncover a potential treatment option for human patients.

T cell-specific gp130 signaling regulates T cells and antibody responses after acute LCMV infection

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University of California, San Diego

T cell fates are heavily dictated by the surrounding cytokine milieu; however the specific contributions of different cytokine families in the context of an infectious environment are incompletely understood. Here we sought to evaluate the role of T cell signaling via gp130, the common co-receptor for the IL-6 family of cytokines, during an acute viral infection. For that we infected CD4-cre x gp130fl/fl mice with LCMV ARM and determined different aspects of adaptive immune responses. We observed that the quantity of gp130-deficient virus-specific CD4 T cells were reduced after the peak of infection and had an altered cytokine profile, producing less IL-21 and more IL-2. Despite unaltered differentiation of T follicular helper (Tfh) cells, Tfh cells expressed less maf, il21, and ICOS. This was accompanied with diminished germinal centers, a dramatic reduction in plasmablast numbers and compromised IgG2 switch. We also found skewing of the virus-specific CD8 T cell compartment away from the CD127+ memory precursor phenotype and towards the KLRG1+ effector phenotype, suggesting a defect in memory development. Consistently, virus-specific CD8 T cells demonstrated a clear reduced capacity to expand after secondary challenge. Overall our data show that signaling through the gp130 pathway in T cells promotes CD8 T cell memory as well as the accumulation and function of virus specific CD4 T cells, which in turn has significant effects on downstream humoral immunity.

Enhancer landscapes revealing CD8+ T cell fate decision during bacterial infection

Bingfei Yu, Ananda Goldrath

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In response to acute infections, CD8+ T cells undergo expansion and differentiation to effector and memory precursor populations, mediating the clearance of pathogens and long-term protection against re-infection. CD8+ T cell fate commitment occurs at effector stage and cell surface markers KLRG1 and CD127 are used to distinguish two CD8+ effector T cell subsets with distinct memory potential: terminally differentiated-effector (KLRG1hiCD127lo) and memory precursor-effector (KLRG1loCD127hi) CD8+ T cells. Transcriptional regulation of CD8+ T cell fate decision has been extensively studied. However, the epigenetic mechanism underlying this cell fate decision is largely unknown. Here, we first generate genome-wide chromatin state maps revealing putative enhancers of terminally differentiated- and memory precursor-effector CD8+ T cell subsets during Listeria monocytogenes infection. We have identified and classified enhancers into active, intermediate and inactive enhancers. Functional annotation of enhancers of both subsets suggest that apoptosis-related genes are required to be activated and repressed in terminally differentiated- and memory precursor-effector CD8+ T cells respectively. Further analysis of de novo motifs enriched in enhancers recovers potential transcription factors that might regulate CD8+ T cells differentiation by binding to enhancers.

Presenter: Zhen, Anjie

HIV-specific immunity derived from chimeric antigen receptor-engineered stem cells

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HIV-1 specific cytotoxic T lymphocytes (CTL) are the key host immune response to HIV infection in controlling viral replication and are an important therapeutic target. We have previously engineered HIV-specific CTL responses through the use of molecularly cloned T cell receptors (TCRs). However, generation of HIV-specific CTL responses for therapy is limited by technical barriers such as varying human leukocyte antigen (HLA) restrictions between different individuals and potential for viral escape by sequence variation. The use of a chimeric T cell receptor containing CD4 linked to the signal domain of the T cell receptor zeta chain (universal T cell receptor) can circumvent these barriers. CD4 chimeric antigen receptor (CD4 CAR) modified T cells inhibit viral replication and kill HIV infected cells in vitro and were reported to have prolonged survival in vivo. However, CD4 CAR modified T cells are susceptible to HIV infection resulting in a limited anti-HIV effect from CD4 CAR modified T cells. Here we report the use of a protective chimeric antigen receptor (CAR) in a hematopoietic stem/ progenitor cell (HSPC) based approach to engineer HIV immunity. We determined that CAR-modified HSPCs differentiate into functional T cells as well as natural killer (NK) cells in vivo in humanized mice and these cells are resistant to HIV infection and suppress HIV replication. These results strongly suggest that stem cell based gene therapy with a CAR may be feasible and effective in treating chronic HIV infection and other morbidities.