Presenter: Achek, Asma

Screening of TLR4 inhibiting peptides using phage display technique

Asma Achek, Hyeon-Jun Shin and Sangdun Choi

Department of Molecular Science and Technology, Ajou University, Suwon, Korea

Toll-like receptors (TLRs) are type I transmembrane proteins that are among the most important receptors in innate immunity. They play a crucial role in inflammatory response upon infection. Since the activation of these receptors protects the body from invading pathogens, several studies have focused on the screening of TLRs-targeting molecules. In the present study, we screened multiple libraries of random peptides against TLR4/MD2 complex and identified several peptides. Among these, one peptide (Ajou-2) that antagonizes TLR4 can be a good candidate for TLR4-mediated diseases. The antagonistic effects of Ajou-2 were confirmed through the inhibition of LPS-induced NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) activation, the suppression of MAPKs (mitogen-activated protein kinases) activation as well as the inhibition of pro-inflammatory cytokines (interleukin 6 and tumor necrosis factor-α). This peptide also inhibited the oxidative stress mediators by downregulating inducible nitric oxide synthase and cyclooxygenase 2 and decreasing the intracellular production of NO and reactive oxygen species. This peptide did not affect TLR2/1, TLR2/6 and TLR7/8/9-mediated inflammatory responses in RAW264.7 cells when co-treated with their cognate ligands. Our results showed that Ajou-2 may be a promising and specific therapeutic option for TLR4-mediated inflammatory diseases.

Presenter: Akkaya, Munir

Toll-like receptor 9 signaling antagonizes the B cell receptor-dependent ability of B cells to process and present antigen to helper T cells.

Munir Akkaya, Billur Akkaya, Ann S Kim, Pietro Miozzo, Haewon Sohn, Mirna Pena, Susan K. Pierce
NIH/NIAID

B cells express both an adaptive antigen-specific B cell receptor (BCR) and innate Toll-like receptors (TLRs) allowing the functional outcome of the B cells' engagement with antigen to be modulated in response to pathogen-derived TLR ligands. In T cell-dependent antibody (Ab) responses, the BCR both signals for B cell proliferation and differentiation and also internalizes bound antigen for processing and presentation to helper T cells (TH cells). Key events in T cell-dependent Ab responses in vivo are dependent on B cell presentation to TH cells. Ab responses are initiated in secondary lymphoid organs by the stable interaction of antigen-primed TH cells with activated antigen-specific B cells through MHC-class II peptide complexes presented on the B cell surface. Dependent, in part, on the quality of the B cell- TH cells interaction, B cells either enter germinal centers (GCs) or differentiate into either short-lived plasma cells (PCs) or GC-independent memory B cells (MBCs). Within the GC, B cells proliferate, somatically hypermutate and subsequently undergo antigen-dependent affinity selection. Selection is dependent on the ability of B cells to capture, process and present antigen to TH cells an event that ultimately results in the differentiation of GC B cells to long-lived MBCs and PCs. Here we show that although the TLR9 ligand, CpG, does not affect early antigen-driven BCR signaling, CpG alters the outcome of BCR signaling, resulting in a unique transcriptional profile, enhanced proliferation and differentiation to PCs. Remarkably, CpG dramatically limits the ability of B cells to process and present antigen to TH cells. In the presence of CpG, BCR-induced upregulation of the expression of CD86 and MHC class II is antagonized and antigen internalized by the BCR is not properly trafficked to antigen processing compartments resulting in reduced numbers of peptide-MHC class II complexes on the B cell surface. Indeed, CpG treated antigen-specific B cells show a reduced ability to maintain contact with antigen-specific T cells and to activate antigen-specific helper T cell proliferation in vitro. These results indicate that TLR9 activation of B cells in T cell-dependent Ab responses would drive B cell toward proliferation and antibody secretion and away from events that are highly dependent on the ability of B cells to present antigen to helper T cells and produce long-lived MBCs and PCs.

Presenter: Akkaya, Billur

Antigen-specific T regulatory cells (Tregs) inhibit dendritic cell (DC) function by capturing peptide-MHCII complexes from the DC surface

Billur Akkaya, Munir Akkaya, Amanda H. Holstein, Olena Kamenyeva, Juraj Kabat, Ethan M. Shevach

NIAID

Tregs are a small fraction of T cells that express the transcription factor Foxp3 and are critical mediators of peripheral immune tolerance. There have been several mechanisms described for Treg mediated suppression of T effector cell (Teff) responses; however, the exact mode of inhibition is largely unclear. A majority of the mechanisms utilized by antigen-specific Tregs to suppress immune responses, suggest an initial inhibition of dendritic cell (DC) function that results in diminished activation of T effector cells (Teff) specific for the homologous target as well as diminished activation of other antigen-specific Teff cells that recognize different antigens presented by the same DC (bystander suppression). In an adoptive transfer model in vivo, we demonstrated that OTII transgenic iTregs specific for Ovalbumin323-339 markedly inhibited the expansion of OTII Teff, but did not inhibit the expansion of co-transferred SMARTA Teff (specific for LCMV NP61-76) even though the transferred DCs displayed both peptides. Analysis of iTreg-DC co-cultures in vitro using flow cytometry and confocal microscopy demonstrated that specific peptide-MHCII complexes were captured and internalized by iTregs and nTregs leaving DCs with decreased levels of antigen. Polyclonal iTregs, naive, and activated antigen-specific Teff did not capture peptide-MHCII complexes to the same extent with antigen specific iTregs. Intravital microscopy of the popliteal lymph node showed that adoptively transferred OTII iTregs made larger clusters around the DC for a longer period of time compared to activated Teff which resulted in a decreased synapse formation between the DCs and co-transferred naive OTII Teff cells.Taken together this study suggests that antigen-specific Tregs inhibit immune responses locally in an antigen-dependent fashion by forming firm interactions with DCs leading to a stripping of peptide-MHCII complexes from the DC surface by a transendocytosis-based mechanism.

Presenter: Anguera, Montserrat

Unusual maintenance of X-chromosome Inactivation predisposes female lymphocytes for increased expression from the inactive X

Montserrat Anguera, Jianle Wang, Camille Syrett, Marianne C. Kramer, Arindam Basu, Vishal Sindhava, Michael Atchison

University of Pennsylvania

Females mount stronger immune responses and clear pathogens faster than males, yet they are more susceptible to autoimmune and inflammatory disorders. One important biological factor underlying this sex bias is the X-chromosome, which contains many immunity-related genes. Female mammals use X-Chromosome Inactivation (XCI) to generate a transcriptionally silent inactive X chromosome enriched with heterochromatic modifications and XIST RNA, which equalizes gene expression between the sexes. We have discovered that XCI is maintained differently in female lymphocytes from mice and humans, where XIST RNA and heterochromatic modifications dynamically associate with the inactive X-chromosome. Mature naive T and B cells are missing XIST RNA and heterochromatin marks on the inactive X, and these marks return to most cells with in vitro stimulation. The disappearance of Xist RNA from the inactive X occurs during lymphocyte development, as common lymphoid progenitors differentiate into pro-B cells in bone marrow. Single-cell RNA FISH analyses of healthy female lymphocytes revealed that the X-linked immunity genes CD40LG, TRL7, and CXCR3 are biallelically-expressed in some naive and activated cells, consistent with the euchromatic features for the inactive X. We found that the relocalization of Xist RNA back to the inactive X in activated lymphocytes requires the transcription factor YY1, capable of binding both RNA and DNA. YY1 deletion in activated splenic B cells disrupts Xist RNA localization to the inactive X and prevents chromosome-wide enrichment of the heterochromatic marks H3K27me3 and H2A-ubiquitin. The return of Xist RNA and heterochromatin modifications to the inactive X occurs during the first cell cycle following splenic B cell activation, with Xist RNA enrichment preceding H3K27me3 deposition on the chromosome. Furthermore, we examined T and B cells from patients with systemic lupus erythematosus, an autoimmune disorder with a strong female bias, and observed mislocalized XIST RNA transcripts and evidence of biallelic expression of immunity-related genes from both X-chromosomes. We propose that the Xi in female lymphocytes is predisposed to become partially reactivated and to overexpress immunity-related genes, providing the first mechanistic evidence for the female-specific enhanced immune responses and increased susceptibility for autoimmunity.

Presenter: Beyaz, Semir

Dietary regulation of tumor immune surveillance in the intestine

Semir Beyaz, Khristian Bauer-Rowe, Michael E Xifaras, Stuart H Orkin, Omer H Yilmaz

Harvard Medical School, Massachusetts Institute of Technology

Pro-obesity diets such as high fat diets (HFD) increase intestinal tumorigenesis in part by elevating the numbers and function of intestinal stem cell (ISC), which are the cells-of-origin for many intestinal tumors. Although the interaction between tumor and immune system plays a critical role in tumor progression, little is known about how a HFD regulates cancer cell intrinsic and extrinsic immune mechanisms during intestinal tumorigenesis. Here, we found that ISCs express high levels of MHC-II on their cell surface implicating a crosstalk between CD4+ T cells and ISCs in immune surveillance. A HFD led to significant downregulation of genes involved in MHC-II pathway, co-stimulation and inflammatory response in ISCs independent of obesity indicating less inflammatory cytokine milieu in the microenvironment of ISCs in mice fed with a HFD. Intestinal adaptation to a HFD consisted of reduction in bacterial diversity and immune infiltration of the crypt. Pharmacological inhibition of JAK/STAT pathway, antibiotics treatment and germ-free mice recapitulated the effects of a HFD on the intestine. Notably, adenomas that arise in a HFD have less MHC-II expression and loss of MHC-II expression in pre-malignant ISCs increased intestinal tumor incidence compared to MHC-II sufficient counterparts. These findings highlight how a HFD alters the immune recognition of ISCs and how such changes contribute to the development of intestinal tumors.

Presenter: Bing, So Jin

GM-CSF plays a pathogenic role in autoimmunity to the neuroretina in the absence of Th1 and Th17 lineage cytokines

So Jin Bing, Phyllis B. Silver, Reiko Horai, Yingyos Jittayasothorn, Rachel R. Caspi

Laboratory of Immunology, NEI, National Institutes of Health, Bethesda, MD, USA

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Presenter: Casson, Cierra

DAP12 promotes resolution of allergic airway inflammation

Cierra Casson, Jessica Hamerman

Benaroya Research Institute

Asthma is a chronic respiratory disease that affects over 300 million people and is increasing in prevalence worldwide. Allergic asthma, the most prevalent form of asthma, is driven by chronic, antigen-dependent airway inflammation that develops following an aberrant response to common environmental stimuli. This allergic airway inflammation causes the wheezing, coughing, and shortness of breath that are typical symptoms of asthma. Though many of the mechanisms that contribute to the initiation of allergic airway inflammation have been defined, strikingly little is known about how airway inflammation is resolved to limit disease. Our data show that the signaling adaptor DAP12 is required for resolution of airway inflammation in response to cockroach antigen (CRA), one of the most common human airway allergens. In the absence of DAP12, mice had increased eosinophil numbers in the airway space following CRA sensitization. Additionally, mRNA encoding the type-2 cytokines IL-5 and IL-13 were increased in the lungs of Dap12-/- mice compared to wild-type controls. Though innate lymphoid cells (ILC2) are thought to be the major producers of IL-5 and IL-13 during allergic inflammation, ILC2 do not express DAP12. Instead, DAP12 is highly expressed by myeloid cells and natural killer cells. Early during sensitization with CRA, we observed an expansion of CD11b+ myeloid cells in Dap12-/- mice. The presence of these CD11b+ myeloid cells corresponded with an increase in IL-33 mRNA in Dap12-/- lungs, a cytokine known to induce IL-5 and IL-13 production. We propose that DAP12 expression in CD11b+ myeloid cells limits IL-33 production, either directly from myeloid cells or indirectly via interactions with the epithelium. This dampening of IL-33 production through DAP12 likely restricts IL-5 and IL-13 production to promote resolution of airway inflammation. In addition to having increased type-2 inflammation after sensitization with CRA, Dap12-/- mice also had increased eosinophil numbers in the BALF in response to challenge with CRA 17 days-post sensitization. These data suggest that DAP12 is not only required to resolve airway inflammation after acute sensitization with allergens, but that an inability to resolve initial inflammation in response to sensitization may lead to exacerbated allergic inflammation upon re-exposure to these allergens. Thus, we have identified DAP12 as a novel factor important for the resolution of allergic airway inflammation.

Presenter: Chang, ZeNan

Novel chimeric antigen receptor converts soluble TGF-[b] into a potent T-cell stimulant

ZeNan Chang, Michael H. Lorenzini, Eugenia Zah, Uyen Tran, Yvonne Y. Chen
University of California, Los Angeles

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Presenter: Chang, Yu-Ling

Crohn's Disease-associated microbes produces metabolites to regulate Human T cell responses

Yu-Ling Chang, Yu-Ling Chang, Maura Rossetti, Gemalene Sunga, David Casero, Jonathan Jacobs, Jonathan Braun

University of California, Los Angeles

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Presenter: Chen, Jasmine

Equine Immune Response to Recombinant Proteins of Corynebacterium pseudotuberculosis

Jasmine Chen, Catherine Conlon, Connie Li, Pari Vanjara, Daniel Crowley, Karen Molinder, and Roberta Pollock

Occidental College

Pigeon fever, also known as dryland distemper, is an equine disease caused by Corynebacterium pseudotuberculosis. This bacterium is a gram-positive pleomorphic intracellular, facultative anaerobe with worldwide distribution. No vaccine is currently available. The three different forms of pigeon fever include external abscesses, internal abscesses, and ulcerative lymphangitis. Horses with internal infections have a relatively high mortality rate of around 40% and need antibiotic treatment, while horses with external abscesses have a low mortality rate and often are not treated with antibiotics. Since horses with internal abscesses do not have unique, specific clinical symptoms, a diagnostic assay is especially important, so that treatment is not delayed. A major goal of our lab is to develop an effective diagnostic assay for pigeon fever. Utilizing western blotting, ELISAs, and recombinant proteins, we are examining differences in the antibody response to C. pseudotuberculosis proteins by horses with varying manifestations of pigeon fever. Initial work testing different cell fractions suggested that this approach is feasible. Current experiments include testing the usefulness of recombinant proteins identified by mass spectrometry and subsequently cloned. To date we have identified, cloned, and tested two immunogenic proteins, phospholipase D (PLD), and trehalose corynomycolyl transferase C (TCTC). Phospholipase D is a major exotoxin that increases vascular permeability by cleaving sphingomyelin and is believed to facilitate spread of infection in the host. Trehalose corynomycolyl transferase C is essential for biosynthesis of corynomycolic acid, a cell wall component of the CMN (Corynebacterium, Mycobacterium and Nocardia) genera.

Presenter: Conlon, Catherine

Developing a vaccine for pigeon fever

Catherine Conlon, Jasmine Chen, Christopher Hino, Daniel Crowley, Dr. Karen Molinder, Dr. Roberta Pollock

Occidental College

Corynebacterium pseudotuberculosis is a gram-positive, intracellular pathogenic bacterium that causes a disease called pigeon fever in horses. Pigeon fever is characterized by painful pus-filled abscesses in subcutaneous tissue (external abscesses), leg lymphatic vessels (ulcerative lymphangitis), and internal organs (internal abscesses). Despite the high prevalence of the disease and high mortality in the internal form, there are no successful equine vaccines currently in existence. Therefore, it is imperative to focus on vaccine development and optimization. We utilize a mouse model for these studies. We tested four potential vaccine components: wildtype equi phospholipase D (PLD); mutant equi PLD; trehalose corynomycolyl transferase C (TCTC); and *C. pseudotuberculosis* cell lysate; using a novel tricomponent adjuvant. PLD is an exotoxin and the crucial virulence factor of *C. pseudotuberculosis*. TCTC is a protein involved in cell wall assembly and maintenance. BALB/c mice were immunized twice with one of the potential vaccine components and challenged with 10^6 *C. pseudotuberculosis* CFU per mouse. The WT equi PLD vaccine gave partial protection (58% survival) while the other vaccines gave low protection (25% or less survival) against the bacteria. All of the potential vaccines showed increase survival compared to mice given adjuvant only (8% survival). WT equi PLD combined with this novel tricomponent may be promising as a vaccine, especially if combined with other *C. pseudotuberculosis* proteins.

Presenter: Covarrubias, Sergio

Long Non-Coding RNA GAPLINC Connects Macrophage Differentiation To Inflammation

Sergio Covarrubias, Ran Song, Kasthuribai Viswanathan, Sol Katzman, Edward K. Wakeland, Susan Carpenter

UC Santa Cruz

Macrophages are critical effector cells of the innate immune system essential for controlling infection and maintaining tissue homeostasis. Perturbations to these signaling pathways can have devastating consequences, leading to diseases, such as Rheumatoid Arthritis and Cancer. Macrophages arise from monocytes in a differentiation process that is tightly regulated, involving many microRNAs, proteins and stage-specific expression of transcription factors. Long non-coding RNAs (lncRNAs) represent the largest group of RNA produced from the genome and are described as transcripts greater than 200 nucleotides in length that lack protein-coding ability. LncRNAs are rapidly emerging as critical regulators of a broad range of biological processes including genomic imprinting, development, and cancer. We sought to identify novel lncRNAs involved in monocyte to macrophage differentiation. We generated comprehensive RNA-sequencing data sets from primary healthy human monocytes and differentiated macrophages and identified hundreds of lncRNAs differentially expressed during differentiation. We characterized one lncRNA, called GAPLINC, which is dramatically induced over one thousand fold transitioning from monocyte to macrophages. Interestingly, this lncRNA is rapidly downregulated upon Toll-like receptor (TLR) stimulation suggesting a connection to inflammatory pathways. Knockding down GAPLINC in primary human macrophages results in an enrichment for inflammation-related genes in our top upregulated genes, suggesting this lncRNA may negatively regulate inflammatory pathways. GAPLINC is localized to the cytoplasm of macrophages bit it does not associate with polysomes. We are in the initial stages of understanding the mechanism by which GAPLINC is functioning in macrophages. Here we reveal an interesting connection between the regulation of macrophage differentiation and the downstream inflammation pathways.

Presenter: de Jesus Carrion, Steven

Control of Tumor Growth by TSLP During Colorectal Cancer

Steven de Jesus Carrion, Steven Ziegler

Benaroya Research Institute

Thymic stromal lymphopoietin (TSLP) is a cytokine involved in promoting tumor growth during breast and pancreatic cancer by promoting Th2 cell-mediated inflammation. Despite being essential to maintain Treg cell homeostasis in the normal mouse and human intestine, a role for TSLP in colorectal cancer has never been shown. To determine if TSLP affects tumor growth during colorectal cancer, we utilized a murine model of colitis-associated colorectal cancer. TSLP deficient mice exhibited decreased tumor numbers when compared to the WT littermate controls. Moreover, we found that tumors in TSLP-/- mice were also significantly smaller in size than WT mouse tumors. TSLP was mainly localized in the tumor tissue, indicating that it might be produced by cancer or cancer-associated cells. Further, TSLP receptor (TSLPR) expression was significantly upregulated after development of cancer. To determine if TSLP signals directly on the tumor cells, we utilized

VillincreTSLPRflox mice in which the TSLPR expression is absent only in intestinal epithelial cells (IECs). Consistent with the TSLP-/- mice, lack of TSLP signaling on IECs led to a decrease in tumor number as well as tumor size, suggesting that TSLP signals directly on the IECs to promote tumor growth. Overall, these data show a novel role for TSLP in controlling tumor progression during colorectal cancer and identify it as a potential target for immunotherapy intervention.

Presenter: Dean, Tantin

Transcription factor Oct1 and cofactor OCA-B control memory and autoimmune T cell responses

Tantin Dean, Arvind Shakya, Jeremy Snook, Stephanie Meek, Heejoo Kim, Laura Dickey, Daniel J. Doty, Robert S. Fujinami, Thomas E. Lane, Matthew A. Williams

University of Utah School of Medicine

The transcriptional co-regulator OCA-B is induced in stimulated primary CD4 T cells, where it docks with its cognate transcription factor Oct1 to directly recognize and regulate critical genes among them Il2, Il21, Il4, Il2ra (Cd25), Ifng, Icos, Ctla4, Csf2(Gmcsf), Tnfrsf4 (Ox40), Tbx21 (Tbet), Stat3 and Stat5a. Importantly, OCA-B's effects only manifest when stimulated cells are rested and restimulated. Consequently, Oct1-/- and Ocab-/- naive CD4 T cell responses to primary stimulation are normal but secondary stimulation results in target gene expression defects of 100-fold or more. Further, both OCA-B and Oct1 are required for CD4 memory formation and response to re-challenge in vivo. We show here that CD4 cells with higher propensity to enter the memory pool express significantly more OCA-B. We also show that OCA-B and Oct1 regulate cytokine target gene expression in the same manner in CD8 as in CD4 cells, and that loss of these factors in T cells results in hypersensitivity and lethality following chronic infection with LCMV clone 13. Lastly, we show that Oct1 loss in T cells is protective in an EAE model, generating fewer highly pathogenic IFNγ+IL17-A+ double-producer cells.

Presenter: Delpoux, Arnaud

Crucial role of FOXO1 in inflationary CD8+ T-cell responses during persistent viral infection

Arnaud Delpoux, Arnaud Delpoux, Rodrigo Hess Michelini, Shilpi Verma, Brittney Wellisch, ChenYen Lai, Chris A. Benedict, Stephen M Hedrick

UCSD

Cytomegalovirus (CMV) establish persistent infection that induce the accumulation of virus-specific T-cells over time in a process called memory inflation. Although persistence of antigen (Ag) is considered essential, the molecular pathway for the inflationary T-cell development is poorly understood. Mainly, inflationary CD8 T-cells have an effector-memory phenotype (KLRG1hi CD27-), but a low percentage of them express a central-memory phenotype (KLRG1lo CD27+), which are considered as their memory precursors. To study the role of FOXO1 in CD8 T cells during a persistent viral infection, we analyzed mouse bone marrow chimeras in which FOXO1 was specifically deleted in CD8 T cells. These mice were infected with MCMV-m157 strain, and the expansion and phenotype of inflationary and acute-contracting T cells was examined. The results revealed that the IE3 and M38 CD8 T-cell responses do not inflate in absence of FOXO1. Furthermore, with an absence of FOXO1, there were fewer Ag-specific CD8 T cells that produced both IFNγ and TNFα. Consistent with this lack of CD8 effector cells the mice were less able to control the virus in the spleen and the liver at day 6 after infection. Moreover, we found that FOXO1 KO Ag-specific CD8 T cells fail to up-regulate the memory associated transcription factors, TCF-1 and EOMES, correlating with a lower percentage of memory precursors (KLRG1lo CD27+). Then, we found that FOXO1 KO Ag-specific CD8 T cells cycle normally (Ki67+), but display an anergic state, as measured by calcium mobilization activation, and are prone to apoptosis. Finally, we delete FOXO1 at day 40 during the infection, we showed that the FOXO1 KO Ag-specific CD8 T cells survive less efficiently and lose their memory characteristics as well as their recall capacities. Collectively, these results demonstrate an intrinsic role for FOXO1 in establishing and maintaining the inflationary memory program that is essential to forming long-lived effector and memory cells. In addition, a memory phenotype is not a permanent differentiation state, it has to be actively maintained by the continued activity of FOXO1.

Presenter: DeRiso, Elizabeth

Novel recruitment of NEMO to TCR microclusters during T cell activation

Elizabeth DeRiso, Andrea Workman, Lawrence P. Kane, and Stephen C. Bunnell

Tufts University Sackler School of Graduate Biomedical Science

Recent advancements in the world of immune therapy for cancer treatment take advantage of T cell signaling controls, highlighting the importance of fully understanding basic signaling mechanisms for therapeutic targeted use. The NFκB essential modulator protein (NEMO), also known as IKKγ, regulates canonical activation of the cell survival and growth transcription factor NFκB, downstream of the T cell receptor (TCR). Patients with mutated or deficient NEMO protein generally have some form of immune deficiency. Despite this important role, however, little is known about the specific mechanisms regulating the sub-cellular localization of NEMO during immunoreceptor signaling. Through high-resolution, dynamic imaging analysis in primary human PBMCs, and Jurkat T cells, we demonstrate that NEMO is recruited into functionally relevant, ubiquitin-dependent microclusters that coincide with TCR-associated kinase Zap70. Additionally, NEMO is recruited into mobile vesicles and larger, slow moving, membrane-bound macroclusters. Our studies indicate that entrance of NEMO into microclusters also depends on Zap70 kinase activity, yet is independent of other proximal signal molecules such as SLP76 and LAT. Finally, recruitment of NEMO into Zap70 clusters occurs within five minutes of TCR engagement, indicating rapid recruitment of NEMO to the TCR. Shifting from the assumed cytosolic localization of NEMO, our results indicate a novel recruitment pathway for NEMO into functionally relevant, TCR-associated microclusters required for NFκB activation.

Presenter: Dolina, Joseph S.

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

Joseph S. Dolina, Zbigniew Mikulski, Joey Lee, and Stephen P. Schoenberger

La Jolla Institute for Allergy and Immunology

It has long been appreciated, but not understood, that the CD8+ cytotoxic T lymphocyte (CTL) dependence on CD4+ T cell help (Th) is conditional; needed for some immunogens but not others. One explanation for this phenomenon envisions Th requirement as an intrinsic property of the pathogen itself rather than its introduction to the immune system. Here we show that dependence of the optimal CD8+ T cell response to Listeria monocytogenes (Lm) on CD4+ T cells is a function of the immunogen dose used for priming, with low dose Lm (LD; 50 or 10^3 CFU WT or ActA, respectively) inducing a primary antigen-specific CTL response profoundly dependent on CD4+ Th cells while that induced by high dose Lm (HD; 4Ã—10^3 or 10^6 CFU WT or ActA, respectively) is significantly inhibited by CD4+CD25+FoxP3+ regulatory T cells (Treg). The Th-independence of HD immunization is not overcome by additional antigen but instead involves the inflammatory response to more bacteria. Evaluation of various TLR pathways as the relevant sensing mechanism showed that TLR2 is required for CTL responses to LD immunization, and that HD immunization in the absence of TLR9 results in a simultaneous loss of CD25+FoxP3+CD4+ Treg cells and increase in conventional CD4+ Th cells and CTLs. Our data thus reveal that the CTL response to the same pathogen is determined by distinct roles for CD4+ T cells as helpers versus regulators based on immunogen dose and demonstrate a previously undescribed role for TLR9 in the regulation of CD4+ Th and Treg cells.

Presenter: Duggan, Jeffrey

BCAP inhibits proliferation and differentiation of myeloid progenitors at the steady state and during demand situations

Jeffrey Duggan, Matthew B. Buechler, Rebecca M. Olson, Tobias M. Hohl, Jessica A. Hamerman

Benaroya Research Institute, University of Washington

B cell adaptor for PI3-kinase (BCAP) is a signaling adaptor expressed in mature hematopoietic cells including monocytes and neutrophils. Here we investigated the role of BCAP in the homeostasis and development of these myeloid lineages. BCAP-/- mice had more bone marrow (BM) monocytes than WT mice, and in mixed WT:BCAP-/- BM chimeras, monocytes and neutrophils skewed towards BCAP-/- origin, showing a competitive advantage for BCAP-/- myeloid cells. BCAP was expressed in bone marrow hematopoietic progenitors, including LSK (Lineage-Sca1+cKit+), CMP (Common Myeloid Progenitor) and GMP (Granulocyte/Macrophage Progenitor) cells. At the steady state, BCAP-/- GMP expressed more IRF8 and less CEBPa than WT GMP, which correlated with an increase in monocyte progenitors and a decrease in granulocyte progenitors amongst GMP. Strikingly, BCAP-/- progenitors proliferated and produced more myeloid cells of both neutrophil and monocyte/macrophage lineages than WT progenitors in myeloid colony forming unit (CFU) assays, supporting a cell-intrinsic role of BCAP in inhibiting myeloid proliferation and differentiation. Consistent with these findings, during cyclophosphamide-induced myeloablation or specific monocyte depletion, BCAP-/- mice replenished circulating monocytes and neutrophils earlier than WT mice. During myeloid replenishment after cyclophosphamide-induced myeloablation, BCAP-/- mice had increased LSK proliferation, and increased numbers of LSK and GMP cells, compared to WT mice. Furthermore, BCAP-/- mice accumulated more monocytes and neutrophils in the spleen than WT mice during Listeria monocytogenes infection. Together, these data identify BCAP as a novel inhibitor of myelopoiesis in the steady state and of emergency myelopoiesis during demand conditions.

Presenter: Dunai, Cordelia

Opposing roles of natural killer cell subsets in a mouse model of acute myeloid leukemia and hematopoietic stem cell transplant

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

UC Davis

Natural killer (NK) cells are lymphocytes that bridge innate and adaptive immune responses. The activity of NK cells is controlled by the integration of activating and inhibitory signals. NK cells can be divided into subsets termed licensed and unlicensed based on the expression of inhibitory receptors with varying affinity for MHC class I molecules. In general, licensed NK cells have higher cytotoxic potential than unlicensed NK cells. Hematopoietic stem cell transplant (HSCT) is a treatment for a number of hematological malignancies, including acute myeloid leukemia (AML). Patients who are not eligible to receive intensive cytoreductive therapy, are at risk for primary refractory disease, or are experiencing relapse are all candidates for HSCT. NK cells are the first lymphocytes to recover post-HSCT and have been shown to be critical for an anti-leukemia response. Here we investigate the contribution of NK cell subsets to anti-leukemia responses in the context of a mouse model of HSCT.

Presenter: Felix, Krysta

Segmented Filamentous Bacteria Confer Protection Against a Lung Infection Through Innate Mechanisms.

Krysta Felix, Nhan Tran, Debdut Naskar, Walid Raslan, and Hsin-Jung Joyce Wu

University of Arizona

Segmented Filamentous Bacteria (SFB), commensal bacteria that colonize the small intestine under normal, healthy conditions, profoundly impact the host immune system. In particular, they induce protection against intestinal pathogens such as *Citrobacter rodentium* and *Entamoeba histolytica*, potentially in an IL-17-dependent manner. Immune responses initiated in the intestines can extend to other mucosal sites, such as the respiratory system. Because of this, we hypothesized that SFB colonization would increase resistance to a lung infection caused by *Streptococcus pneumoniae*. We found that SFB colonization does not make a difference in disease susceptibility in WT mice. We further identified B cells as key players in protecting mice against *S. pneumoniae* infection. In the absence of B cells, mice that lack SFB develop more severe disease, while those that are colonized by SFB maintain resistance, as demonstrated by protection against body temperature decrease and weight loss, as well as decreased lung bacterial load and lower neutrophil infiltration. These data suggest that SFB protect the host against *S. pneumoniae* infection in a manner dependent on the innate immune system. In a B cell transfer model, B cell-mediated protection requires B cell-intrinsic MyD88, as in contrast to mice receiving WT B cells, mice receiving MyD88-/- B cells are unable to maintain normal temperature after infection, a phenotype comparable to total lack of lymphocytes. Our data demonstrate the significant impact of the gut microbiota on infections even in gut-distal locations such as the lungs.

Presenter: Ganesan, Anusha Preethi

Tissue-resident memory features are linked to the magnitude of cytotoxic T cell responses in human lung cancer

Anusha Preethi Ganesan1, 2, Oliver Wood3, Eva M Garrido-Martin4, Serena Chee3, Toby Mellows4, James Clarke3, Daniela Samaniego-Castruita1, Divya Singh1, Gregory Seumois1, Aiman Alzetani5, Edwin Woo5, Peter S Friedmann4, Gareth J Thomas3, Emma V King3, Tilman Sanchez-Elsner4, Pandurangan Vijayanand1,4,6, Christian H Ottensmeier 3,5,6.

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Presenter: Gao, Yajing

Discover T cell fate in a dish: generation and profiling of pathogen-specific CD4 T cells

Yajing Gao, Edward K Wakeland, Chandrashekhar Pasare

University of Texas Southwestern Medical Center

CD4 T cells are vital for optimal functioning of the adaptive immune system. In addition to secreting effectors that have direct effects, they are critical for CD8 T cell and B cell responses. Naive CD4 T cells are activated by antigen-presenting cells (APCs, especially dendritic cells) and differentiate into distinct effector subtypes, distinguished by their profiles of cytokine production. CD4 T cell effector cytokines such as IFNγ, IL-17A and IL-13 are specialized and critical for elimination of specific spectrum of pathogens. Therefore, CD4 T cell response, depending on the nature of the invading enemy, is rather evolved to generate an optimal proportion of each subsets in order to provide effective host protection. The pathogen-specificity of effector CD4 T cells hence is a combination of TCR recognition of pathogen epitopes and the customized wiring of effector functions. So far little is known about the exact mechanisms by which the later specificity is determined. Here we describe an in vitro method to generate and trace pathogen-specific CD4 T cells, allowing us to unbiasedly assess pathogen-directed clonal expansion and differentiation of naive CD4 T cells. Utilizing this system, we have been able to prime pathogen-specific CD4 T cells in vitro, which as a result exhibit cytokine profiles consistent with the established in vivo response for a given pathogen. Adoptive-transfer of these CD4 T cells to a naive mouse, followed by pathogen challenge, showed specificity towards the priming pathogen. Using fate-mapping and transcriptional profiling, we found evidence of heterogeneity and specificity between Th17 subsets generated by different pathogenic stimuli. Collectively, our results suggest an effective way to study pathogen specificity of T cell differentiation and we are rigorously using this tool to define early programming that imprints terminal effector function on primed CD4 T cells.

Presenter: Gosliner, Stanley

Identification of Potential Biomarkers for Different Forms of Corynebacterium pseudotuberculosis Infections

Stanley Gosliner, Connie Li, Cheryl Okumura, Yu Gao, Roberta Pollock, Karen Molinder

Occidental College

Corynebacterium pseudotuberculosis is a gram positive bacterium that infects horses, causing a disease commonly known as pigeon fever. Pigeon fever causes the development of painful abscesses that typically manifest externally, but can also manifest internally, which is the more lethal form of the disease. Currently, there is no quick and effective method to diagnose internal abscesses, which often leads to delayed treatment resulting in a high mortality rate. We set out to identify *C. pseudotuberculosis* protein products that are uniquely recognized by serum from horses with either internal or external abscesses. The identification of proteins that are uniquely expressed in just one form of the disease is the first step in developing diagnostic tests to effectively differentiate between those forms. We used immunoprecipitation to isolate potential proteins of interest. Antibodies from horses with either internal or external abscesses were crosslinked to protein G beads. These antibodies were then incubated with *C. pseudotuberculosis* cell lysate and the bound proteins eluted. The collected proteins were analyzed by Coomassie staining, Western blot, and tandem mass spectrometry. Western blot analysis alone did not yield results that were effective in distinguishing between the two forms of the disease. Therefore, we used tandem mass spectrometry to analyze the collected protein samples. Through multidimensional protein identification technology (MudPIT), we observed differences in the proteins recognized by horses with different forms of the disease. While there was variability between horses, proteins specific to each form of the disease were identified and will be cloned for further analysis.

Presenter: Guthmiller, Jenna

B cell-intrinsic and extrinsic IL-10 signaling are critical for the generation of germinal center B cells and protective anti-Plasmodium humoral immunity

Jenna Guthmiller, Amy Graham, Ryan Zander, Rosemary Pope, Noah Butler

University of Oklahoma Health Sciences Center

Plasmodium parasites cause over 200 million cases of malaria and nearly half a million deaths each year. Clinical and experimental studies show that humoral immunity is essential for Plasmodium parasite control and clearance. However, naturally acquired humoral immunity is short-lived and non-sterilizing, leaving individuals susceptible to repeated episodes of malarial disease. The mechanisms that skew B cell activity towards short-lived, inefficient humoral immune reactions are not fully understood. Recent reports highlight that excessive IFN-γ exacerbates malarial disease by limiting germinal center (GC) responses and that IL-10 can limit severe malarial disease by suppressing the activity of IFN-γ. Here we test the hypothesis that Plasmodium infection-induced IL-10 and IFN-γ directly counter-regulate B cell function and anti-Plasmodium humoral immunity. We found that direct IL-10 signaling in B cells is essential for GC B cell responses, parasite-specific IgG secretion, parasite clearance, and host survival. By contrast, IFN-γ signaling in B cells directly triggers non-protective, short-lived plasmablast responses and limits GC B cell responses resulting in reduced secreted parasite-specific IgG. Thus, IL-10 both directly and indirectly, by antagonizing IFN-γ, promotes GC B cell development and anti-Plasmodium humoral immunity. We further identified that both B cell-intrinsic IL-10 signaling and IL-10-mediated suppression of IFN-γ limit T-bet expression in B cells. Genetic deletion of T-bet in B cells abrogates short-lived plasmablast responses, promotes robust GC B cell responses, and markedly enhances parasite-specific IgG secretion. Collectively, our data identify that B cell-intrinsic IL-10 and IFN-γ signaling counter-regulate expression of T-bet, which acts as a molecular switch to promote either short-lived plasmablast responses or limit GC B cell responses. Our findings encourage reconsideration of IFN-γ and IL-10 as correlates of protection and pathology during clinical malaria.

Presenter: Gutierrez-Franco, Jorge

B7-H6, a new ligand for the activating NK cell receptor NKp30, is present in exosome-enriched fractions of serum samples from pregnant women

Jorge Gutierrez-Franco, Rodolfo Hernandez-Gutierrez, Hector Alatorre-Guillen, Rosa Elena Navarro-Hernandez, Marta Escarra-Senmarti, Gloria Yareli Gutierrez-Silerio, Alejandra Natali Vega MagaÃ±a, Miriam Ruth Bueno-Topete, Susana del Toro-Arreola

Instituto de Enfermedades CrÃ³nico-Degenerativas, Departamento de BiologÃa Molecular y GenÃ³mica, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara. Guadalajara, Jalisco, MÃ©xico

INTRODUCTION. A challenging question in pregnancy is why the fetal-placental unit is not rejected by the maternal immune system. Many mechanisms have been proposed to explain this immune tolerance. For instance, elevated soluble MIC, which is capable of down-regulating the activating receptor NKG2D on PBMC, has been seen in pregnant women. Different isoforms of NKp30, another NK-activating receptor, have been associated with preeclampsia and spontaneous abortion. B7-H6 is a cell-surface ligand for NKp30 and soluble forms have been seen in pathological conditions including cancer. However, the nature of B7-H6 has been largely unexplored during pregnancy. OBJECTIVE. In this present work, we aimed on the characterization of soluble B7-H6 during the development of normal human pregnancy. MATERIALS AND METHODS. Serum samples were obtained from healthy pregnant during the first (n= 36), second (n=17), and third trimester (n=17). Healthy non-pregnant female of reproductive age served as control (n=30). B7-H6 was revealed by Western blotting. A further characterization was performed through an immunoproteomic approach based on 2DE-Western blotting combined with MALDI-MS. Additionally, a comercial exosome isolation reagent (from serum) was used to search for B7-H6 in exosomal fractions. RESULTS. In all pregnant women, we observed the presence of two new bands of B7-H6 with lower than the reported MW of 51 kDa. These bands were either a heavy (~37 kDa) or a light isoform (~30 kDa) and were mutually exclusive. B7-H6 was maintained throughout pregnancy. The confirmation of the identity and molecular mass of each isoform indicates that B7-H6, while maintaining the C- and N-termini, is most likely being released during pregnancy by an alternative mechanism to proteolytic cleavage. We found that the isoforms of B7-H6 were released as exosomes and directly in extracelullar environment. CONCLUSIONS. Soluble B7-H6 is constitutively expressed during pregnancy. Moreover, presented two new isoforms, which were mainly liberated by exosomes, though B7-H6 was also found in exosome-depleted serum.

Presenter: Han, Hongwei

IL-33 promotes gastrointestinal allergy in a TSLP-independent manner

Hongwei Han, Florence Roan, Laura K. Johnston, Paul J. Bryce, Steven F. Ziegler

Benaroya Research Institute

Not to be placed on website

Presenter: Hatton, Olivia

NKG2A-Expressing Natural Killer Cells Dominate the Response to Autologous Lymphoblastoid Cells Infected with Epstein-Barr Virus

Olivia Hatton, Dara Strauss-Albee, Nancy Q. Zhao, Mikel D. Haggadone, Judith Shanika Pelpola, Sheri M. Krams, Olivia M. Martinez, Catherine A. Blish

Colorado College

Epstein-Barr virus (EBV) is a human γ-herpesvirus that establishes latency and lifelong infection in host B cells while achieving a balance with the host immune response. When the immune system is perturbed through immunosuppression or immunodeficiency, however, these latently-infected B cells can give rise to aggressive B cell lymphomas. Natural killer (NK) cells are regarded as critical in the early immune response to viral infection, but their role in controlling expansion of infected B cells is not understood. Here we report that NK cells from healthy human donors display increased killing of autologous B lymphoblastoid cell lines (LCL) harboring latent EBV compared to primary B cells. Co-culture of NK cells with autologous EBV+ LCL identifies an NK cell population that produces IFNγ and mobilizes the cytotoxic granule protein CD107a. Multi-parameter flow cytometry and Boolean analysis reveals that these functional cells are enriched for expression of the NK cell receptor NKG2A. Further, NKG2A+ NK cells more efficiently lyse autologous LCL than do NKG2A- NK cells. More specifically, NKG2A+2B4+CD16-CD57-NKG2C-NKG2D+ cells constitute the predominant NK cell population that responds to latently-infected autologous EBV+ B cells. Thus, a subset of NK cells is enhanced for the ability to recognize and eliminate autologous, EBV-infected transformed cells, laying the groundwork for harnessing this subset for therapeutic use in EBV+ malignancies.

Presenter: Hobbs, Sam

Tissue-resident memory CD8+ T cells promote local antigen specific inflammation

Sam Hobbs, Jeffrey C. Nolz

Oregon Health and Science University

Tissue-resident memory (Trm) CD8+ T cells permanently reside in non-lymphoid tissues where they are important mediators of both host defense and inflammatory disease. In particular, Trm cells have been implicated in multiple skin inflammatory diseases including psoriasis and allergic contact dermatitis. Here, we sought to model contact-dependent inflammation of the skin by topical application of antigen to the site of Trm formation. We show that stimulation of resting Trm cells with antigen results in a local inflammatory response that is characterized by swelling and recruitment of leukocytes into the skin. Using IFNγ-YFP reporter TCR transgenic CD8+ T cells, we are able to identify Trm cells exhibiting local effector function within hours of stimulation with antigen. CD8+ T cells can produce inflammatory cytokines including IFNγ and TNFα, which could increase the expression of adhesion molecules on the skin-associated vascular endothelium. In fact, administration of P- and E- selectin blocking antibodies reduced both swelling and leukocyte recruitment into the skin following peptide challenge. Importantly, inflammatory responses did not occur in skin lacking Trm cells, was not affected by the depletion of circulating memory CD8+ T cells, and was antigen specific. Finally, we show that repeated challenges of the skin with antigen resulted in substantially increased numbers of CD69+ antigen-specific Trm cells. These results demonstrate that Trm CD8+ T cells mediate antigen-specific inflammation and expand locally during repeated exposures to cognate antigen, which could have implications for the treatment or prevention of inflammatory disorders of the skin.

Presenter: Hopp, Christine S.

Longitudinal analysis of Plasmodium falciparum-specific atypical and classical memory B cell responses to natural malaria infection in children and adults

Christine S. Hopp, Akshay T. Krishnamurty, Silvia Portugal, Chris Thouvenel, Ogobara K. Doumbo, Boubacar Traore, Susan K. Pierce, David J. Rawlings, Marion Pepper, Peter D. Crompton

NIH, NIAID

Not to be placed on website

Presenter: Horai, Reiko

Vitamin A deficiency impacts acquisition, but not expression, of autoimmunity to the neuroretina

Reiko Horai, Ru Zhou, So Jin Bing, Kaska Wloka, Jun Chen, Phyllis B. Silver, Yingyos Jittayasothorn, Rachel R. Caspi

National Eye Institute, National Institutes of Health

Vitamin A (VitA) and its derivative retinoic acid are essential for immunological responses. Acquisition of effector responses is impeded in VitA deficient (VAD) mice. However, little is known about maintenance and expression of previously acquired effector function in the VAD environment or its impact on progression of autoimmune diseases. We examined this using two models of uveitis: experimental autoimmune uveitis (EAU) induced by active immunization and spontaneous uveitis in retina-specific T cell receptor transgenic (R161H) mice, and in the model of experimental autoimmune encephalomyelitis (EAE). VAD was induced by dietary lack of VitA from before birth, or by daily injections of a pan-retinoic acid receptor inhibitor BMS493 in adult mice fed normal chow. VAD mice were essentially resistant to induction of EAU or EAE and displayed impaired effector T cell responses. Defective priming/acquisition of effector function by VAD T cells was also evident in vitro. Interestingly, however, effector T cells primed in a VitA-sufficient environment were able to function in VAD recipients, as evidenced by maintenance of high lineage-specific effector cytokine production and induction of EAU. Furthermore, spontaneously uveitic R161H mice fed with VAD diet, in which priming of pathogenic T cells had occurs before onset of full VAD, appeared to develop unreduced or even exacerbated spontaneous uveitis compared to VitA-sufficient R161H mice. We conclude that although priming of naive T cells in the VAD environment is defective, effector function acquired under VAD sufficient conditions is maintained and can be expressed under VAD conditions. Because dietary lack of VitA is rarely profound and may be seasonal, our findings may shed light on immunity and autoimmunity in geographical regions where dietary VitA is limiting.

Presenter: Hsu, Lih-Yun

A Hypomorphic Allele of ZAP-70 reveals the importance of T effector function in the progression of autoimmune disease

Lih-Yun Hsu, Debra Cheng1,3, Jesse E. Jun2 , Arthur Weiss1,2,3

1. Department of Medicine, 2. Department of Anatomy, 3. Rosalind Russell and Ephraim Engleman Rheumatology Research Center, 4. Howard Hughes Medical Institute, University of California San Francisco, San Francisco, CA 94143

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Presenter: Hudig, Dorothy

Individual Differences in Human Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC): Correlations with Phenotypic Properties and CD16A V&F Genotypes

Dorothy Hudig, Jennifer J-J. Tang, Alexander P. Sung, Michael J. Guglielmo, Doug Redelman, Julie Smith-Gagen, Suzanne Vernon, Lucinda Bateman, Isabel Barao

University of Nevada Reno School of Medicine

Antibody-dependent cell-mediated cytotoxicity (ADCC) supports anti-viral protection and monoclonal antibody anti-tumor therapies. We used two assays to compare ADCC of healthy donors: CX@1:1 (the percentage cells killed at a 1:1 ratio of CD16A positive NK cells to target cells that were pre-labeled with saturating antibody); and EC50 (the effective concentration of antibody that supports 50% of maximal ADCC). CX@1:1 measures lytic capacity while the EC50 measures cellular recognition; there was no correlation between the 2 assays though we observed 4.5-fold differences among donors in each assay. We correlated ADCC with 5 cytometric parameters (the median fluorescent intensities [MFIs] of the CD16A IgG Fc-receptor; the %CD2positive NK cells & MFIs of the adhesion molecule CD2; the MFIs of perforin; and the %CD16Apositive of CD56positive NK cells) and with CD16A V&F genotypes that affect FcR affinity. For CX@1:1, the best donor killed 73% of targets while the worst killed 16% and perforin levels correlated weakly ($P=0.11$). Unexpectedly the %CD16Apositive cells among NK cells correlated ($P<0.05$). CD16A and CD2 were dissociated from CX@1:1 as might be expected for the high antibody concentrations. For EC50s, there were no statistically significant correlations except for the expected difference between the EC50s of donors with V/V &V/F vs. F/F genotypes, which had a weak correlation ($P=0.11$). We conclude that substantial individual differences in ADCC per CD16A positive NK cells are determined by parameters other than cell surface receptors and perforin. Differences in intracellular signaling among CD16A NK cells within the donors could contribute to the differences in ADCC.

Presenter: Jain, Aakanksha

Innate control of memory CD4 T cell effector function

Aakanksha Jain, Aakanksha Jain, Chandrashekhar Pasare

University of Texas Southwestern Medical Center

Pathogen recognition by DCs via activation of pattern recognition receptors results in the up-regulation of MHC and co-stimulatory molecules and secretion of pro-inflammatory cytokines. While naive CD4 T cells require all three of these signals from DCs for successful activation and differentiation, MHC-TCR interaction is thought to be sufficient for memory CD4 T cell reactivation. Surprisingly, we find that reactivation of memory CD4 T cells via TCR alone induces significantly less effector cytokine production as compared to DC mediated reactivation suggesting that additional innate signals still play a role. Further investigation revealed a requirement for IL-1β dependent signaling in addition to TCR activation for optimal cytokine production by reactivated Th17 cells. This is consistent with previous studies showing that lack of IL-1R on T cells leads to defective Th17 responses. Interestingly, here we find that IL-1R deficient mice harbored similar proportions of T cells committed to the Th17 lineage as measured by Rorγt expression and IL-17A production. Instead, T cell intrinsic IL-1R signaling was required for Th17 cytokine secretion upon reactivation of polyclonal or antigen specific memory CD4 T cells from secondary lymphoid organs. We obtained similar results from in vivo reactivation of lamina propria resident Th17 cells highlighting the global requirement for IL-1R signaling for Th17 effector function irrespective of their tissue of residence. Collectively, our data point to a novel innate controlled regulation of memory Th17 cell function and warrants revisiting the currently established paradigm for memory CD4 T cell reactivation.

Presenter: Kastenschmidt, Jenna M.

The role of group 2 innate lymphoid cells in the pathogenesis of mdx skeletal muscle

Jenna M. Kastenschmidt, Ileen Avetyan, S. Armando Villalta

University of California, Irvine

Group 2 innate lymphoid cells (ILC2s) are critical regulators of type 2 inflammatory responses, and function to promote tissue repair and restoration of homeostasis. However, whether these repair processes include regenerative responses that encompass ILC2 and tissue stem cell interactions is largely unknown. To address this question, we examined the role of ILC2s in regulating muscle regeneration, a tissue with a high regenerative capacity that is dependent on a well-defined muscle stem cell population (i.e. satellite cells). We show that ILC2s are activated and numbers are elevated in skeletal muscle of mdx mice, a model of Duchenne muscular dystrophy (DMD) in which the regulation of disease pathogenesis is controlled in part by the balance between type I and type II immune responses. Gain-of-function studies using IL-2/anti-IL2 complex (IL-2c) showed that this treatment effectively increased the number of ILC2s in mdx skeletal muscle and increased myofiber cross sectional area, suggesting enhanced muscle regeneration. In addition, ablation of IL-13-producing cells, including ILC2s, inhibited the recruitment of muscle eosinophils, an innate immune cell population previously implicated in muscle regeneration. Collectively our data support a working model in which ILC2s ameliorate the pathogenesis of muscular dystrophy by promoting muscle regeneration, likely through the regulation of satellite cell function and type II inflammatory responses that promote skeletal muscle regeneration.

Presenter: Knaub, Alynna

Activation-induced chromatin decondensation in the lymphoid lineage

Alynna Knaub, Sierra McDonald, Jason S. Rawlings

Furman University

It has previously been demonstrated that chromatin decondensation is required for αβ T cell proliferation. B cells, γδ T cells, and Natural Killer cells share the same lineage as αβ T cells, originating from hematopoietic stem cells and a common lymphoid progenitor. In this study, we investigated the hypothesis that chromatin decondensation is required for proliferation of these other cells of the lymphoid lineage. We assessed chromatin decondensation by using Western Blot to test for Histone H3 solubility and Flow Cytometry to test Histone H3 accessibility. We show that Lipopolysaccharide, a known activator of B cells, induces the decondensation of chromatin in B cells. To determine if both T and B cells can decondense chromatin at the same rate, both were stimulated with PMA and Ionomycin. B cells and αβ T cells decondense chromatin in a similar manner, although there appears to be differences in kinetics between the two. Preliminary data collected on NK cells and γδ T cells suggest that their rate of decondensation is similar to αβ T cells in response to PMA and Ionomycin stimulation. Recently, our lab demonstrated that calcium is required and sufficient to initiate chromatin decondensation in αβ T cells, future studies will investigate if calcium can also regulate the decondensation of B cells, γδ T cells, and NK cells, establishing a conserved mechanism to control proliferation of the lymphoid lineage.

Presenter: Kornbluth, Richard

Multimeric Soluble 4-1BBL as a T Cell Stimulator for Adoptive Immunotherapy

Richard Kornbluth, Victoria S. Hamilton, and Geoffrey W. Stone

Multimeric Biotherapeutics, Inc.

Members of the TNF SuperFamily of ligands (TNFSFs) have significant potential as immuno-oncology agents. The TNFSFs are trimeric membrane proteins that can be cleaved into soluble single trimers. While the soluble single trimers can be easily prepared and studied, they have little or no activity in vivo. This deficiency is caused by the need to cluster their cognate receptors in the plane of the membrane in order to induce a supramolecular signaling complex on the cytoplasmic side of the plasma membrane. For the TNFSF ligands, this requires that they be used as many-trimer multimers that mimic the natural expression of many trimers on the surface of stimulating cells. To meet this need, we prepared fusion proteins comprised of the extracellular domains of TNFSF ligands joined to a natural protein that provides a multimerization scaffold. When surfactant protein D (SP-D) is used as a scaffold, the result is a 4-trimer TNFSF ligand product (UltraLigands). Our published studies have described such multimeric forms of CD40L, OX40L, GITRL, CD27L/CD70, BAFF, RANKL, and TRAIL and shown that they are highly active in vitro and in vivo. As an extension of this work, 4-trimer forms of murine and human 4-1BBL (CD137L, TNFSF9) were constructed and expressed in CHO cells. As a co-stimulatory molecule, SP-D-4-1BBL (Ultra4-1BBL) activated both CD4+ and CD8+ T cells in vitro. Given the interest in 4-1BB (CD137) as a marker of therapeutically effective tumor-infiltrating lymphocytes (TILs), SP-D-4-1BBL should be a useful should be a useful growth factor for TIL manufacturing and T cell culturing in general.

Presenter: Kovar, Marek

Strong sustained IL-2 signal selectively targeted to CD25+ cells dramatically increases sensitivity to LPS

*Marek Kovar, Jakub Tomala, Petra Weberova, Barbara Tomalova and Marek Kovar**

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IL-2 is potent immunostimulatory molecule which plays a key role in T and NK cell activation and expansion, however, it is also an essential cytokine for homeostasis of Treg cells. IL-2 exerts its pleiotropic activities through binding either to dimeric receptor composed from IL-2Rβ (CD122) and common cytokine receptor gamma chain (γc, CD132) or to trimeric receptor composed from IL-2Rα (CD25), IL-2Rβ and γc. CD25 has been termed the "low-affinity" IL-2R (Kd~10 nM) and it is not involved in signal transduction. A dimer of CD122 and CD132 binds IL-2 with intermediate affinity (Kd~1 nM) and is present on CD122high populations, namely memory CD8+ T cells (CD3+CD8+CD44highCD122high) and NK cells (CD3-NK1.1+DX5+). A complex of CD25, CD122 and CD132 binds IL-2 with high affinity (Kd~10 pM) and it is present on CD25high populations, namely activated T and Treg cells (CD3+CD4+CD25+Foxp3+). It was reported that in vivo biological activity of IL-2 can be dramatically increased by association of IL-2 with anti-IL-2 mAbs and that these IL-2 complexes possess selective stimulatory activity determined by the clone of anti-IL-2 mAb used. IL-2/S4B6 mAb complexes were described to be highly stimulatory for NK and memory CD8+ T cells and intermediately also for Treg cells. IL-2/JES6-1 mAb complexes are stimulatory solely for CD25high cells. We have found recently that mice treated with IL-2/JES6-1 mAb complexes (3 daily doses of 1.5 μg IL-2 equivalent) show dramatically increased sensitivity to LPS-mediated shock and mortality (~10-30 times). Mice treated with IL-2/JES6-1 mAb complexes and challenged with 10 μg LPS possess 5-10 times higher plasma concentration of TNF-α (90 min. after LPS challenge) in comparison to control mice challenged with 200 μg LPS. Interestingly, IL-2/S4B6 complexes almost do not sensitize mice to LPS.

Acknowledgement: This work was supported by grant 13-12885S from Czech Science Foundation and by LQ1604 NPU II provided by MEYS, CZ.1.O5/1.1.00/02.0109 BIOCEV provided by ERDF and MEYS and Institutional Research Concept RVO 61388971.

Presenter: Kranich, Jan

Immune-complex independent Targeting of Native Antigen to Follicular Dendritic Cells accelerates humoral immune responses

Jan Kranich, Ashreta Latha, Agnieska Foltyn-Arfa Kia, Lisa Rausch and Thomas Brocker

LMU Munich

Not to be placed on website.

Presenter: Kuan, Emma

Thymic stromal lymphopoietin promotes interplay between tumor cells and myeloid cells to regulate breast tumor progression

Emma Kuan, Steven Ziegler

Benaroya Research Institute

The cytokine thymic stromal lymphopoietin (TSLP) has been implicated in controlling various human cancer development through regulating Th2 responses by directly acting on CD4+ T cells. However, it is still largely unknown the role of TSLP in other cell types within tumors since TSLP receptor (TSLPR) is widely expressed on many cell types, including many hematopoietic cells and epithelial cells under inflammatory conditions. We found that TSLP signaling in both human and mouse breast tumor cells is important to maintain their survival and their capacity to produce TSLP in vitro. By using murine metastatic breast tumor models we found that the lack of TSLP in hematopoietic cells or TSLPR, but interestingly not TSLP, in breast tumor cells, leads to significantly smaller primary tumor size. We identified the critical TSLP source is myeloid cells within tumors. We also showed that asthmatic mice with TSLP locally expressing in lungs leads to more tumor metastasis to lungs and TSLP blockage in lungs significantly reduced tumor metastasis. Besides tumor cells, we discovered that TSLP signaling in Ly6Chi monocytes is also crucial for promoting tumor progression by regulating monocyte suppressor functions and their ability to differentiate into tumor associated macrophages. Our work is the first to show myeloid cell derived-TSLP plays an important role in directly promoting breast tumor progression via maintaining tumor cell survival. We also provide another novel mechanism of the requirement of TSLP signaling in regulating the pro-tumor functions in Ly6Chi monocytes. These studies define a novel TSLP-mediated crosstalk between tumor-infiltrating myeloid cells and tumor cells and provide an effective therapeutic intervention in metastatic breast cancer.

Presenter: Kurd, Nadia

Factors that influence autoreactive thymocyte fate

Nadia Kurd, Ashley Hoover, Jenny Yoon, Ellen Robey

University of California Berkeley

The processes of negative selection and agonist selection both make important contributions to establishing central tolerance. However, the factors that determine whether an autoreactive thymocyte will undergo negative selection or agonist selection following antigen encounter remain ambiguous. Here, we make use of a thymic slice system in which class I-restricted thymocytes undergo both negative selection and agonist selection to investigate these factors. We demonstrate that interactions with phagocytic antigen presenting cells (APCs) promote negative selection, and highlight a previously unappreciated role for phagocytes as “executioners” of autoreactive thymocytes. In contrast, the cytokine IL-15, which is produced by thymic epithelial cells, protects against death and promotes agonist selection. Taken together, our data suggest that the ability of a thymic APC to phagocytose or deliver cytokines could be a key factor in determining the fate of an autoreactive thymocyte.

Presenter: Lai, Jen-Feng

CD11c+ Interstitial Macrophages promote TSLP-mediated acute allergic airway inflammation independent of DC and T cell priming in the lymph nodes

Jen-Feng Lai, Lucas Thompson, Steven Ziegler

Benaroya Research Institute

Not to be placed on website.

Presenter: Lam, Wing Y

Mitochondrial Pyruvate Import Promotes Long- Term Survival of Antibody-Secreting Plasma Cells

Wing Y Lam, Amy M. Becker, Krista M. Kennerly, Rachel Wong, Jonathan D. Curtis, Elizabeth M. Payne, Kyle S. McCommis, Johannes Fahrmann, Hannah A. Pizzato, Ryan M. Nunley, Jieun Lee, Michael J. Wolfgang, Gary J. Patti, Brian N. Finck, Erika L. Pearce, and Deepta Bhattacharya

Washington University in St.Louis School of Medicine

Durable antibody production after vaccination or infection is mediated by long-lived plasma cells (LLPCs). Pathways that specifically allow LLPCs to persist remain unknown. Through bioenergetic profiling, we found that human and mouse LLPCs could robustly engage pyruvate-dependent respiration whereas their short-lived counterparts could not. LLPCs took up more glucose than did short-lived plasma cells (SLPCs) in vivo, and this glucose was essential for the generation of pyruvate. Glucose was primarily used to glycosylate antibodies, but glycolysis could be promoted by stimuli such as low ATP levels and the resultant pyruvate used for respiration by LLPCs. Deletion of Mpc2, which encodes an essential component of the mitochondrial pyruvate carrier, led to a progressive loss of LLPCs and loss of vaccine-specific antibodies in vivo. Thus, glucose uptake and mitochondrial pyruvate import prevent bioenergetic crises and allow LLPCs to provide enduring antibody-mediated immunity.

Presenter: Lane, Ryan

Distinct functions of hematopoietic and nonhematopoietic PD-L1 on the antitumor CD8+ T cell response

Ryan Lane, Amanda W. Lund

Oregon Health & Science University

PD-L1 has been an effective target for immunotherapy to treat metastatic melanoma. While it is assumed that tumor and myeloid cell-specific PD-L1 induce CD8 T cell exhaustion in the tumor, other cells in the tumor microenvironment also express PD-L1 and their role in T cell exhaustion has not been ruled out. We have identified novel PD-L1 expression on nonhematopoietic components of the tumor microenvironment, including lymphatic endothelial cells (LECs), blood endothelial cells (BECs), and fibroblasts, indicating a potential role in local CD8 T cell suppression. Importantly, in chronic LCMV infection nonhematopoietic PD-L1 controls local T cell function to prevent immunopathology and hematopoietic PD-L1 suppresses T cell activation. In this work we test the hypothesis that, similar to LCMV infection, nonhematopoietic PD-L1 suppresses local T cell function in the tumor microenvironment and hematopoietic PD-L1 limits CD8 T cell activation. Here we use B16F10 murine melanoma to investigate the role of PD-L1 on the antitumor immune response by implantation into PD-L1 KO mice. In tumor-bearing animals, endogenous CD8 T cells had increased numbers and markers of activation (CD44, core-2 O-linked glycosylation, and PD-1) in tumor draining lymph nodes (LN) and spleens of PD-L1 KO compared to WT mice. Additionally, tumor infiltrating CD8 T cells demonstrate increased IFNg production upon 3 hr ex vivo stimulation with PMA/ionomycin in PD-L1 KO mice compared to WT controls. To determine the contribution of hematopoietic and nonhematopoietic PD-L1 to either the reduced activation in secondary lymphoid organs or rather the impaired cytotoxic function in the tumor microenvironment we generated PD-L1 KO bone marrow chimeras. Mice lacking hematopoietic PD-L1 (PD-L1 KO-->WT) have increased CD8 T cell counts in tumor draining LNs and increased markers of activation, compared to WT-->WT controls. Importantly, mice lacking either hematopoietic (PD-L1 KO-->WT) or nonhematopoietic PD-L1 (WT-->PD-L1 KO) had increased numbers of CD8 tumor infiltrates as well as increased activation markers compared to WT-->WT controls. This work indicates that hematopoietic cell PD-L1 may limit antitumor T cell expansion in lymph nodes as well as local CD8 T cell function, and that tissue resident nonhematopoietic cells may suppress local antitumor effector T cell function.

Presenter: Meermeier, Erin W.

Human TRAV1-2-negative MR1-restricted T cells detect S. pyogenes and alternatives to MAIT riboflavin-based antigens

Erin W. Meermeier, Aneta Worley, Bruno F. Laugel, Andrew K. Sewell, Alexandra J. Corbett, Jamie Rossjohn, James McCluskey, Melanie J. Harriff, Tamera Franks, Marielle C. Gold & David M. Lewinsohn

Oregon Health and Science University

Mucosal-associated invariant T (MAIT) cells detect microbial antigens presented by the HLA-Ib molecule MR1 through the exclusive use of a TRAV1-2-containing T cell receptor (TCR)α. However, whether other TCRs can recognize MR1-antigen is unknown. Here we use MR1 tetramer staining and ex vivo analysis with mycobacteria-infected MR1-deficient cells to demonstrate the presence of functional human MR1-restricted T cells that lack TRAV1-2. We characterize an MR1-restricted clone that expresses the TRAV12-2 TCRα, which lacks residues previously shown to be critical for MR1-antigen recognition. In contrast to TRAV1-2+ MAIT cells, this TRAV12-2-expressing clone displays a distinct pattern of microbial recognition by detecting infection with the riboflavin auxotroph Streptococcus pyogenes. Thus, MR1-restricted T cells can discriminate between microbes in a TCR-dependent manner. As known MAIT antigens are derived from riboflavin metabolites, this suggests that TRAV12-2+ clone recognizes unique antigens. We provide preliminary evidence that MR1-restricted TCR usage differs between blood and a mucosal tissue site. We postulate that additional MR1-restricted T-cell subsets may play a unique role in defense against infection by broadening the recognition of microbial metabolites.

Presenter: Mittelsteadt, Kristen

ICOS-dependent PI3K signaling in regulatory T cell development and function

Kristen Mittelsteadt, Daniel J. Campbell

University of Washington, Benaroya Research Institute

Foxp3+ regulatory T cells (Treg) are critical for maintaining immune tolerance and preventing inflammatory disease at different tissue sites. The co-stimulatory receptor ICOS is an important regulator of T cell function. Recent work suggests that ICOS signaling is required to control Treg cell abundance and function in vivo in peripheral tissues and sites of inflammation. Although the mechanisms by which ICOS supports peripheral Tregs is unknown, they may involve its ability to potently activate PI3K signaling. In order to study the role of ICOS-dependent PI3K activation in Treg homeostasis and function, we obtained mice that carry a knock-in mutation in the Icos gene that alters the cytoplasmic tail motif of the ICOS protein and specifically abolishes ICOS-dependent PI3K signaling (YF mice). Indeed, Treg frequencies and proliferation are reduced in these mice in lymphoid organs. YF Tregs have impaired regulation of downstream PI3K targets, including decreased phosphorylation of AKT and S6, and altered expression of the lymphoid homing molecule CD62L. In mixed bone marrow chimeras, YF Tregs exhibit a competitive disadvantage in populating non-lymphoid tissue. Furthermore, YF mice are unable to recover from both active and passive transfer experimental autoimmune encephalomyelitis, correlating with lower frequencies of activated Tregs at effector sites. Taken together, we suggest an important role for ICOS-mediated PI3K activation in the maintenance and function of Tregs.

Presenter: Myles, Arpita

Characterization of metabolic profiles in follicular B cells post activation

Arpita Myles, Michael Cancro

University of Pennsylvania

Immune cell activation is accompanied with bioenergetic shifts that support proliferation, growth and differentiation. The metabolic profile of B lymphocytes in response to activation is not well characterized. We report that follicular B cells (CD23+) upregulate their mitochondrial mass upon stimulation through the B cell receptor (BCR) or toll-like receptor 9 (TLR9). This is accompanied by increase in levels of reactive oxygen species (ROS), basal respiration, ATP production and spare respiratory capacity. In vitro, addition of anti-CD40 (to mimic co-stimulatory signals) causes a reduction in glycolysis and mitochondrial respiration. Since B cell differentiation in vivo is tailored by cytokines like IL-4 and IL-21, we examined their effects on metabolic profiles of ex vivo activated follicular B cells. IL-4 reduced mitochondrial metabolism in context of both BCR + anti-CD40 and TLR9 stimulation. IL-21, on the other hand, upregulated metabolism of CpG (TLR9 ligand) treated cells but did not substantially alter properties of BCR + anti-CD40 group. These findings suggest that B cell activation in vitro is accompanied by metabolic shifts, which are tuned by co-stimulatory and cytokine signals. The mechanism underlying these shifts, as well as their functional relevance in the context of B cell differentiation in vivo models of infection and immunization requires further investigation.

Presenter: Nguyen, Minh Quan

The Contribution of T Follicular Regulatory Cells in HIV Persistence

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

UCLA

The HIV reservoir presents a major challenge in finding a functional cure for HIV. In lymphoid tissue, T follicular helper cells in the germinal center (GC) have been previously found to be contributors to the viral reservoir. Recently, a subset of regulatory T cells was identified in the GC of the mouse lymphoid tissues and was named T follicular regulatory cells (TFR). TFR express a similar set of markers to that of T follicular helper cells such as CD4, CXCR5, and BCL6. In addition, they express T regulatory cells markers: FoxP3 and CD25. Our data shows that cells with a TFR phenotype (CD3+, FoxP3+, CXCR5+, BCL6+) are present in the human tonsils and can also be detected in peripheral blood. This leads to our hypothesis in which TFR may play a role in HIV pathogenesis and in the maintenance of the HIV reservoir, as T follicular helper cells are known to form part of the HIV reservoir. It remains unknown whether only T follicular helper cells in the lymph nodes harbor HIV in HIV-infected individuals, or whether TFR present in lymph nodes and peripheral blood also contribute to HIV persistence. Using multicolor flow cytometry, peripheral blood mononuclear cells (PBMC) were stained for intracellular TFR markers: FoxP3 and BCL6, in combination with other cell surface markers including CD4, CD3, CD8, CD25, TIGIT, PD1 and ICOS as well as the HIV co-receptors, CCR5 and CXCR4. We examined the expression profile of peripheral blood T follicular helper cells and TFR in fresh and frozen PBMC from healthy individuals and in frozen PBMC from HIV-infected individuals, obtained from the Multicenter AIDS Cohort Study (MACS). We found that a subset of CD3+CD4+CXCR5+ T cells expresses CD25 and FoxP3 in fresh PBMC. These CD4+CXCR5+CD25+FoxP3+ T cells are identified as TFR, which are distinct from the CXCR5+FoxP3- T follicular helper cells. Unlike the expression profile in lymphoid tissue, BCL6 expression could not be detected in peripheral blood TFR. Thus, peripheral blood TFR may differ from lymphoid tissue TFR in that they do not express BCL6. TFR also expressed CD62L, a lymphoid tissue homing receptor, suggesting that peripheral blood TFR may be the pre-cursors of lymphoid tissue TFR. CD62L expression, however, was found to be unstable upon freezing, so CD62L would not be useful for immunotyping of frozen PBMC, which is currently underway. Lastly, peripheral blood CD4+CXCR5+FoxP3+CD25+ TFR expressed the HIV co-receptors, CCR5 and CXCR4. This indicates that TFR can potentially be infected by CCR5-tropic and CXCR4-tropic HIV and that TFR have the potential to serve as a viral reservoir. Overall, these initial findings haven given insights into the immunophenotype of TFR and demonstrate that these cells have the potential to contribute to HIV persistence. In the future, we will be exploring the expression profile of TFR in HIV-infected individuals. Peripheral TFR are a unique T cell subset that expresses Foxp3 and CD25, in addition to T follicular helper cell markers, and can potentially contribute to HIV persistence.

Presenter: Ong, SuFey

c-Ski mediates Th17 and Treg responses to TGFβ

SuFey Ong, Mathais Hauri-Hohl, Steven F Ziegler

Benaroya Research Institute

TGFβ is involved in both induced Treg and Th17 differentiation via its induction of Foxp3 and RORγt. The oncogene c-ski regulates TGFβ function by inhibiting SMAD2/3 from both binding to SMAD4 and to DNA, repressing the transcription of TGFβ-inducible genes. Previously, we have shown that the absence of ski in medullary thymic epithelial cells (mTECs) limits cellular growth due to unchecked TGFβ signaling. Given that central role of Treg and Th17 cells in the development of autoimmune disease, we explored the role of Ski and TGFβ signaling in T cell function and differentiation. We demonstrate that the absence of Ski in CD4+ T cells leads to amplified TGFβ sensitivity as shown by increased Th17 differentiation at low levels of TGFβ in vitro. Additionally, Foxp3CreSkifl/fl T cells polarized in the presence of TGFβ and IL-2 in vitro lead to increased Treg conversion in culture, suggesting that Ski may also play a role in Treg stability. Both of these strains show decreased EAE pathogenicity compared to control mice. In conclusion, we have shown that Ski functions as a modifier of TGFβ signaling in CD4+ T cells and can directly influence the outcome of autoimmune disease pathogenesis.

Presenter: Osborn, Jossef

Enzymatic synthesis of core 2 O-glycans governs cellular trafficking potential within the memory CD8+ T cell compartment

Jossef Osborn, Jana Mooster, Jeffrey Nolz

Oregon Health & Science University

Synthesis of core 2 O-glycans is essential for memory CD8+ T cells to generate functional ligands for E- and P-selectin, which facilitate extravasation out of the circulation and into non-lymphoid tissues. We have previously demonstrated that memory CD8+ T cells are epigenetically poised to synthesize core 2 O-glycans in response to cytokines including IL-15, in an antigen-independent manner. The circulating memory CD8+ T cell pool is comprised of self-renewing central memory (Tcm) and terminally differentiated, highly cytolytic effector memory (Tem) subsets, but the capacity for these subsets to traffic into non-lymphoid tissues and control infections remains controversial. Herein, we demonstrate that core 2 O-glycan synthesis is highly active in the Tcm CD8+ T cell subset, but not in Tem populations. Signaling through the IL-15 receptor directly controls the ability of memory CD8+ T cell subsets to synthesize core 2 O-glycans and generate functional E- and P-selectin ligands. Finally, using a variety of inflammatory and infection models, we demonstrate that Tcm CD8+ T cells are better than Tem cells at leaving the circulation and entering non-lymphoid tissues, including the skin and lung. These data identify core 2 O-glycan synthesis as a key mechanism that dictates subset-specific memory CD8+ T cell trafficking and demonstrate that Tcm actively enter inflamed non-lymphoid tissues, whereas Tem are essentially excluded.

Presenter: Pizzato, Hannah A.

Mitochondrial Pyruvate Import is Necessary for the Development of GMP-derived Cells

Hannah A. Pizzato, Wing Y. Lam, Brian N. Finck, and Deepta Bhattacharya

Washington University in Saint Louis School of Medicine

As hematopoietic stem cells differentiate, they gradually lose the potential to generate specific blood cell types until commitment to a single lineage is achieved. As hematopoietic stem cells are exceedingly rare, their differentiation occurs concomitantly with a progressive expansion of downstream progenitors to ensure the sufficient production of mature cells. How this expansion occurs remains unknown but may involve metabolic switches. Here we demonstrate that mitochondrial pyruvate deficiency via the deletion of an essential subunit of the mitochondrial pyruvate carrier (Mpc2) leads to a significant reduction in neutrophils and monocytes, yet spares naive B and T cells. This defect begins at the transition between the common myeloid progenitor (CMP) and the granulocyte-monocyte progenitor (GMP). In wild type mice, glucose uptake increases during the CMP to GMP transition, suggesting the existence of an essential metabolic switch dependent on glycolysis and mitochondrial respiration. In contrast, glucose uptake remains relatively low in mature neutrophils. Mpc2-deficient neutrophils can be rescued by retroviral transduction of the long-chain fatty acid transporter Slc27a1, which can provide alternate respiratory substrates and/or compensate for defective fatty acid synthesis. Ongoing work is designed to identify extrinsic cues that enact these metabolic switches. Taken together, these data demonstrate a requirement for mitochondrial pyruvate for the expansion of GMPs and downstream myeloid cells.

Presenter: Pothoven, Kathryn

Neutrophil-derived Oncostatin M is elevated in eosinophilic mucosal disease and may promote epithelial barrier dysfunction through the induction of epithelial mesenchymal transition.

Kathryn Pothoven, James Norton, Lydia Suh, Roderick Carter, Bruce K Tan, Robert P Schleimer

Northwestern University Feinberg School of Medicine

Epithelial barrier dysfunction is thought to play a role in many mucosal diseases including asthma, chronic rhinosinusitis (CRS), and eosinophilic esophagitis (EoE). Oncostatin M (OSM), was elevated in both mRNA (28.3 fold, p<.01, n=12) and protein (4.4 fold, p<.05, n=12-19) in the nasal polyps of CRS patients compared to control tissue. OSM protein was also elevated in induced sputum from asthmatic patients compared to control (7 fold, p<.05, n=11-12). Additionally, OSM mRNA was elevated in esophageal biopsies of EoE patients vs. controls, (3 fold, p<.01). OSM stimulation of differentiated airway epithelium induced barrier dysfunction, measured by decreased transepithelial electrical resistance (63% reduction, p<.0001, n=11) and increased permeability to 10kD dextran (2.45 fold, p<.05, n=5). Levels of OSM protein in tissue lysates from CRS and controls correlated with levels of a2-macroglobulin, a marker of epithelial leak, in matched nasal secretions (r=.5055, p<.01), suggesting that OSM may play a role in epithelial barrier dysfunction in mucosal disease. To determine which cell type was producing OSM, nasal polyp sections were stained for OSM and hematopoetic cell specific markers. OSM showed co-localization with neutrophil elastase (n=10), however OSM did not co-localize with markers for eosinophils, macrophages, T cells or B cells (n=3-5). GM-CSF has previously been shown to induce OSM. GM-CSF protein was elevated in nasal polyps compared to control. GM-CSF was sufficient to induce OSM protein in cell culture supernatants of ex vivo cultured neutrophils. Interestingly, immunofluorescent staining for GM-CSF showed that the source of GM-CSF were the OSM+ neutrophils, suggesting that these neutrophils were driving their own production of OSM (n=5). Additionally, bronchial biopsy sections were stained for OSM and neutrophil elastase. None of the control patients (n-4), 60% of the moderate asthmatics (n=5), and 100% of the severe asthmatics (n=6) had OSM+ neutrophils within the biopsy section, suggesting that neutrophil derived OSM may mediate barrier dysfunction in both CRS and asthma. To determine the mechanism of OSM mediated epithelial barrier dysfunction, we measured mRNA expression of OSM and markers of epithelial mesenchymal transition (EMT). OSM expression positively correlated with mesenchymal phenotype markers, (VIM, SERPINH1, ITGA5, and DDR2), EMT transcription factors, (TWIST1, TWIST2, ZEB1, and ZEB2) and the EMT inducer, TGFB1, suggesting that OSM expression and EMT may be related. OSM stimulation of differentiated airway epithelial cultures induced protein expression of mesenchymal markers, S100A4 and [a]SMA, and cellular proliferation marker ki67 shown through immunofluorescence, suggesting that OSM may mediate barrier dysfunction through the induction of EMT. Therapeutic targeting of OSM, its signaling, mediators of EMT, neutrophils, or GM-CSF may be beneficial in the treatment of eosinophilic mucosal disease.

Presenter: Przybyla, Anna

Defining the activation state of melanoma antigen specific CD8 cells as naive/resting memory or effector cells.

Anna Przybyla, Anna Przybyla, Eliza Kwiatkowska-Borowczyk, Anna Kozlowska, Katarzyna Gryska, Richard Caspell, Andrzej Mackiewicz, Paul V. Lehmann

Poznan University of Medical Sciences, Department of Medical Biotechnology, Poland, CTL Cellular Technology Limited

Naive tumor antigen-specific CD8 cells typically (with few exceptions) occur in very low frequency in blood, and do not secrete IFNg or Granzyme B (GzB). Effector CD8 cells, capable of cytolysis, secrete GzB in addition to IFNg, and due to clonal expansion occur in increased frequency in blood. Resting CD8 memory cells secrete IFNg but not GzB also occurring in increased frequency in blood. Such resting memory cells will re-express GzB within several days upon antigen re-encounter, converting into effector CD8 cells. Using this basic features of CD8 cell biology, we performed IFNg and GzB ELISPOT assays to measure the frequency of melanoma antigen-specific CD8 cells secreting these analytes 24h and 72h after antigen stimulation. Tyrosinase (Tyr), gp100 and Melan/MART-1 antigen peptide pools were tested on PBMC of healthy donors and vaccinated melanoma patients. Of the above melanoma antigens only Tyr triggered relatively high frequency (~1/1000) CD8 cells at 24h ex vivo. At this time point these CD8 cells did not produce GzB yet, however they engaged in GzB production by 72h after antigen stimulation. Therefore, Tyr-specific CD8 cells in healthy controls are clonally expanded resting memory cells (IFNg +/GzB -) that can be reactivated to become effector cells (IFNg +/GzB+) within 72h. We are presently testing whether ex vivo (24h) GzB production is elicited in vaccinated melanoma patients by Tyr revealing the conversion of these memory cells into effector cells due to vaccination, or whether vaccinated subjects show evidence for immunological priming to the other melanoma antigens to which healthy controls do not seem to be primed, using this above time resolved IFNg/GzB approach.

Presenter: Randilea, Nichols

NLRC4 inflammasome activation in neutrophils is sufficient to cause systemic inflammatory disease

Nichols Randilea, Jakob von Moltke, Russell E. Vance

University of California, Berkeley

Not to be placed on website

Presenter: Raychaudhuri, Kumarkrishna

IL-17A inhibits expression of IL-17 lineage cytokines through a negative feedback loop involving IL-24, and controls autoimmune disease

Kumarkrishna Raychaudhuri, Wai Po Chong, Reiko Horai, Phyllis B. Silver, Yingyos Jittayasothorn, Chi-Chao Chan, Jun Chen, Rachel R. Caspi

NEI. NIH

The Th17 response has been associated with autoimmune diseases in patients and in animal models. IL-17A is recognized as the Th17 signature cytokine and IL-17-producing T cells are pathogenic effectors in models of autoimmunity, including experimental autoimmune uveitis (EAU). Paradoxically, injection of IL-17 was shown by others to ameliorate the disease. We used a model of spontaneous uveitis in IRBP T cell receptor transgenic R161H mice to investigate the susceptibility to disease of these mice on an IL-17A-/- background. Surprisingly, IL-17A-/- R161H mice developed essentially undiminished uveitis and IL-17-/- R161H T cells, polarized to Th17 and infused into wild type recipients, induced similar disease to IL-17A sufficient R161H T cells. Interestingly, IL-17A-/- R161H T cells polarized under Th17 conditions produced elevated amounts of other Th17-related cytokines, i.e. IL-17F, GM-CSF and IL-22. Supplementing these cultures with recombinant IL-17A normalized the elevated production of those cytokines. RNAseq analysis revealed that IL-17A-/- T cells displayed lower IL-24 expression compared to their IL-17 sufficient counterparts. Mechanistic studies indicated a negative feedback loop where IL-17A induces Th17 cells to produce IL-24, which suppresses production of Th17 lineage cytokines. Finally, injection of recombinant IL-24 ameliorated adoptive Th17-induced EAU, and conversely, depletion of IL-24 in Th17 cells increased their pathogenicity and elevated disease severity. These data suggest that IL-17A exerts a negative feedback on autopathogenic Th17 cells via IL-24 production, which limits the expression of other Th17 lineage cytokines that may contribute to pathogenicity.

Presenter: Reiner, Gabrielle

Induction of Potent and Durable Anti-Tumor Immunity by Intratumoral Injection of STING-Activating Synthetic Cyclic Dinucleotides

Natalie Surh, Gabrielle Reiner, Laura Hix Glickman1, David B. Kanne1, Kelsey Sivick Gauthier1, George E. Katibah1, Anthony L. Desbien1, Brian Francica2, Justin J. Leong1, Leonard Sung1, Ken Metchette1, Shailaja Kasibhatla3, Jie Li3, Anne Marie Culazzo Pferdekamper3, Lianxing Zheng4, Charles Cho3, Yan Feng4, Jeffery M. McKenna4 John A. Tallarico4, Steven L. Bender3, Chudi Ndubaku1, Sarah M. McWhirter1, Charles Drake2, Thomas F. Gajewski5 and Thomas W. Dubensky, Jr.1; 1Aduro Biotech, Inc., Berkeley, CA; 2Johns Hopkins University, Baltimore MD; 3Genomics Institute of the Novartis Research Foundation, San Diego, CA; 4Novartis Institutes for BioMedical Research, Cambridge MA; 5University of Chicago, Chicago IL

Aduro Biotech

Substantial evidence indicates that tumor infiltrating lymphocytes (TILs) are predictive of a positive clinical outcome in response to immunotherapy. In human melanoma, spontaneous T cell infiltration into the tumor microenvironment (TME) is correlated with a type I interferon (IFN) transcriptional signature. Similarly, in mice bearing melanoma, there is a correlation between expression of IFN-ÃŸ by tumor-resident dendritic cells and priming of tumor-specific immunity. Induction of IFN-ÃŸ in this context is dependent upon activation of Stimulator of Interferon Genes (STING), a critical component of the cytosolic DNA sensing pathway of the innate immune system. STING is activated by binding of cyclic dinucleotides (CDNs) produced by an intracellular enzyme, cyclic GMP-AMP synthase (cGAS), in response to the presence of cytosolic pathogen or tumor-derived DNA. STING induction of type I IFN within the TME leads to the priming and activation of tumor antigen-specific CD8+ T cell immunity. We hypothesized that direct activation of STING in the TME by intratumoral (IT) injection of synthetic CDNs would induce potent anti-tumor immunity against a broad repertoire of an individualâ€™s tumor antigenic milieu. Through screening a panel of synthetic CDNs, we selected ADU-S100 (MIW815) for clinical development for its ability to broadly and potently activate all human STING alleles, elicit profound tumor regression of injected and distal lesions in several aggressive syngeneic mouse tumor models, and promote durable CD8+ T cell-mediated anti-tumor immunity. A bell-shaped ADU-S100 dose response curve was found to delineate regression of injected tumor, induction of tumor-specific CD8+ T cell immunity, and protection against autologous tumor challenge. In vivo mechanistic studies demonstrate that STING-mediated anti-tumor immunity is due in part to an acute pro-inflammatory TNF-a-mediated cytokine response, as well as a tumor-specific CD8+ T cell response. Studies in chimeric wild-type and STING-/- mice showed that while STING signaling in the tumor stromal compartment contributes to acute tumor rejection, STING signaling in the hematopoietic compartment is required for induction of CD8+ T cell mediated anti-tumor immunity. Anti-tumor efficacy is enhanced by combination with immune checkpoint inhibitors, including a-PD-1 and a-CTLA4, informing future clinical development. The ability to elicit innate and T cell-mediated anti-tumor immunity via activation of STING in the TME demonstrates that CDNs have high translational potential for the treatment of patients with advanced/metastatic solid tumors. To this end, a Phase 1 clinical study is in progress to evaluate the safety and tolerability and possible anti-tumor effects in subjects with cutaneously-accessible non UV-induced and UV-induced malignancies or lymphomas given repeated IT doses of ADU-S100 (NCT02675439).

Presenter: Resop, Rachel S.

The effect of HIV-1 infection on S1P-R1 expression and function in thymocytes and their progenitors during entry into and egress from the human thymus

Rachel S. Resop, Joshua Craft, Dimitrios Vatakis, Bianca Blom and Christel H. Uittenbogaart

UCLA

Lack of adequate T cell regeneration in HIV infected individuals is likely due to a defect in the entry of hematopoietic stem cells (HSC) into and egress of naive T cells from the thymus to the periphery. These phenomena remain understudied and not well understood. We studied the effect of HIV-1 infection on the receptors to Sphingosine-1-phosphate (S1P), a chemotactic sphingolipid mediator, during thymocyte trafficking. Our novel findings show that HIV infection changes the expression patterns of S1P-R1, which as we recently reported (Resop et al., JACI 2016) is normally expressed at the mRNA and protein levels only in the most mature CD3hi thymocytes that have lost CD69 (CD3hiCD69-) and are about to exit the thymus. We used multicolor (10+) flow cytometry and Quantitative Real-Time PCR (qRT-PCR) to examine the dynamics of S1P-R1 expression on Hematopoietic Stem Cell (HSC) progenitors and thymocytes during HIV infection. Mice implanted with human fetal thymus/liver (HIS-mice) infected with CXCR4- or CCR5-tropic HIV-1 were injected intravenously with CFSE-labeled HSC to examine the effect of HIV on HSC entry, thymocyte development and egress. We also investigated the mechanism of changes in S1P-R1 and its transcriptional regulator, Kruppel-Like factor 2 (KLF2). We found that S1P-R1 is expressed at low-moderate levels on human HSC. In infected HIS mice CFSE labeled CD34+ progenitors developed into mature thymocytes in the human thymus/liver implant and a subset expressed S1P-R1, indicating that entry into the thymus and development are functional during early HIV infection. Surprisingly, we found that S1P-R1 (as well as KLF2) was significantly upregulated in mature thymocytes post-HIV infection. Intriguingly, S1P-R1 was also upregulated by HIV within the CD3+CD69+ population, which normally does not express S1P-R1. S1P-R1 signaling as measured by pAkt was not impaired in infected thymocytes, which is interesting in the context of published data demonstrating that S1P-R1 response in HIV infection may be impaired in other cell types. Our results show that the mechanism of increased S1P-R1 in the thymus by HIV may be due to cytokines including Interferon-beta (IFN-β), which significantly increased S1P-R1 expression in both CD3hiCD69- and CD3+CD69+ thymocyte subsets treated in vitro with exogenous cytokine. Tumor Necrosis Factor alpha (TNF-α) also significantly increased S1P-R1 mean Fluorescence Intensity (MFI) on thymocytes in vitro. Additional cytokines perturbed during HIV infection are currently under investigation. S1P-R1 upregulation post-HIV-1 infection may offer insight into T cell reconstitution mechanisms during infection and provide potential alternate avenues for immunotherapy.

Presenter: Richer, Martin

IL-10 directly dampens CD8 T cell function via Mgat5-mediated N-glycosylation and galectin 3 binding

Martin Richer, Logan K. Smith, Giselle M. Boukhaled, Stephanie A. Condotta, Alisha Chitrakar, Jenna J. Guthmiller, Noah S. Butler, Connie M. Krawczyk

McGill University and University of Oklahoma Health Sciences Center

Antigen sensitivity, or the ability of CD8 T cells to become activated and acquire effector functions in the presence of low levels of antigen, is critical for cell mediated immunity directed against chronic infections and cancer. Our recent work has established that antigen sensitivity is dictated by cytokines present in the microenvironment during T cell activation. Whether pathogens that establish chronic infection can co-opt these regulatory pathways to counter host immunity has not been addressed. Here we tested the hypothesis that the anti-inflammatory cytokine IL-10 functions early, and independently of PD-1, during chronic infection to dampen the antigen sensitivity of CD8 T cells and facilitate the establishment of viral persistence. We found that IL-10 signaling directly to CD8 T cells enhanced Mgat5-dependent N-glycosylation of CD8 T cell surface proteins. Excess N-glycosylation led to enhanced binding of galectin 3, which reduced T cell receptor (TCR) clustering, TCR signaling capacity, and reduced the antigen sensitivity of CD8 T cells. Strikingly, and despite increases in N-glycosylation, antigen sensitivity, TCR clustering, and CD8 T cell function are restored in mice lacking galectin 3 allowing for better viral control of LCMV clone 13 infections. These data reveal a novel interplay between the chronic inflammatory milieu, the glycosylation machinery, and T cell function in the context of chronic infections and potentially identify novel therapeutic targets to enhance T cell function.

Presenter: Robinson, Elektra

Investigating the Regulatory Role of LncRNA-Aim2 in Mouse Macrophages

Elektra Robinson, Sergio Covarrubias, Vanille J. Greiner, Michael T. McManus, Susan Carpenter

University of California, Santa Cruz

Advancements in next generation sequencing has provided us with an unprecedented view of the human genome. One of the most fascinating findings is that less than 3% of the genome codes for protein coding exons, yet more than 85% of the genome is transcribed. We are now faced with the challenge of understanding what these RNA genes do and whether they play key roles in biological processes. The largest group of RNA produced from the genome is Long noncoding RNA (lncRNA). LncRNAs are described as transcripts greater than 200 nucleotides in length that do not code for protein. To date there is experimental data available on approximately 1% of known lncRNAs. Our lab is investigating how lncRNAs act as key regulators during inflammation and host defense against infection. Here we describe a new inflammatory inducible lncRNA we have identified upstream of the protein coding gene absent in melanoma 2 (Aim2) on chromosome 1. Aim2 is a critical component of the innate immune response to DNA. It is highly activated by the type 1 Interferon (IFN) pathway. Aim2 forms an inflammasome complex in response to cytosolic DNA stimulation and results in the activation and processing of the critical proinflammatory proteins IL1ÃŸ and IL18. We identified lncRNA-Aim2 through RNA-sequencing of murine macrophages. We see that it is co-expressed with Aim2 following inflammatory stimulation. We have successfully used CRISPRi to knockdown expression of lncRNA-Aim2 in macrophages. Our preliminary data shows that lncRNA-Aim2 does not function in cis to regulate Aim2. We are now carrying out RNA-seq to determine if lncRNA-Am2 functions to regulate other immune genes.

Presenter: Rosental, Benyamin

Evolutionary Origin of the Mammalian Hematopoietic System Found in a Colonial Chordate.

Benyamin Rosental, Benyamin Rosental, Mark A. Kowarsky, Daniel M. Corey, Katherine J. Ishizuka, Karla J. Palmeri, Shih-Yu Chen, Rahul Sinha, Jun Seita, Irving L. Weissman, Ayelet Voskoboynik.

Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine.

To gain insight into the evolutionary relationship between vertebrate and invertebrate hematopoietic system, we have characterized the immune system and cell populations of the colonial tunicate Botryllus schlosseri. B. schlosseri belongs to a group considered the closest living invertebrate relative of vertebrates, it has bidirectional blood cell flow through an interconnected vasculature. To isolate and characterize the Botryllus cell populations we adapted Fluorescence-Activated Cell Sorting. We used Cytof Mass Cytometry to scan 50 diverse antibodies. Antibodies that differentially bind to B. schlosseri cells, in combination with lectins and fluorescent reagents activated by enzymes, were used to isolate live B. schlosseri cell types. Additionally, we used mouse serum against the Botryllus Histocompatibility Factor and analysis of cell size, granularity and auto fluorescence to isolate 34 cell populations. We prepared libraries from these populations for RNAseq, and analyzed their gene expression. This analysis revealed cell population homolog to mammalian hematopoietic stem cells, which upon transplantation, migrated to known stem cell niche and differentiated into other cell lineages. Interestingly, we have shown that this niche is homolog to mouse bone marrow stromal cells. Using functional immunological assays for cytotoxicity and phagocytosis we characterized 3 different phagocytic cell-types. One of these demonstrated transcriptional and functional features resembling myeloid cells in vertebrates. Furthermore, we identified a B. schlosseri cytotoxic cell population originating from large granular lymphocyte-like cells. Our data suggests that the common ancestor of tunicates and vertebrates had a true hematopoietic myeloid lineage, while the cytotoxic cells may result from a convergent evolutionary mechanism.

Presenter: Salvador, Ryan

Gut microbiota as a source of signals that trigger spontaneous ocular autoimmunity

Ryan Salvador[1], R Horai[1], CR ZÃ¡rate-BladÃ©s[1], K Itoh[2], Y Jittayasothorn[1], Y Umesaki[3], RR Caspi[1]

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Presenter: Savage, Hannah

Uncovering the phenotype of pathogen-reactive innate-like B cells

Hannah Savage, Nicole Baumgarth

UC Davis

B-1 cells, an innate-like subset of B lymphocytes, generate protective IgM antibodies to many viral and bacterial pathogens. The respective contributions by the two known subsets of B-1 cells, CD5+ (B-1a) and CD5neg (B-1b), to IgM production are unclear. We showed previously that B-1a, but not B-1b cells, migrate to the respiratory tract draining mediastinal lymph node (MedLN) in response to influenza infection, resulting in increased B-1 derived IgM-production at that site. In contrast, following infection with Streptococcus pneumonia, Borrelia hermsii and Salmonella spp, others reported increased secretion of IgM by B-1b cells. Here we aimed to resolve the reasons underlying the apparent discrepancies in responsiveness of B-1a and B-1b cells to the various pathogens. Given the observed restrictions on B-1a migration to the MedLN after influenza infection, we first determined the phenotype of the IgM-secreting B-1 cells in the MedLN. Surprisingly, the results showed that CD5 neg B-1 cells were the main source of B-1-derived IgM on days 3, 5, and 7 after influenza infection, suggesting that CD5 expression of B-1a cells is lost after differentiation to an IgM-secreting cell. In support, in vitro studies demonstrated that three-day cultures of highly FACS-purified B-1a (CD5+) cells stimulated with LPS contained mainly CD5neg cells. This was not due to a preferential outgrowth of contaminating B-1b cells, as B-1a and B-1b cells showed similar rates of cell death, spiking of cultures with B-1b cells did not significantly affect numbers of CD5neg cells, and B-1a cells proliferated even more than B-1b cells when stimulated with LPS. Ongoing studies using chimeras generated with purified B-1a cells will determine whether a similar conversion of B-1a to â€œB-1bâ€ also occurs in vivo after influenza infection. Our data demonstrate that activated B-1a cells lose CD5 expression and become â€œB-1b likeâ€ IgM-secreting cells. The findings suggest that surface phenotype analysis alone is insufficient to conclude on the contributions of B-1a and B-1b cells to IgM production. Furthermore, our results indicate that CD19+ CD43+ CD5neg â€œB-1bâ€cells, currently regarded as a distinct subset of B-1 cells, and attributed to have memory-like functions, are composed, at least in part, of differentiated, IgM-secreting B-1a cells. (Funding: NIH/NIAID R01 AI051354, AI085568 and U19 AI109962, T-32 AI060555, 2T32OD010931-09, 5T35OD010956-14, UL1 TR000002 and linked award TL1 TR000133)

Presenter: Seki, Scott

PKM2 is a critical driver of pathologic immune responses during CNS demyelination

Scott Seki, Max Stevenson, Lelisa Gemta, Vlad Serbulea, Norbert Leitinger, Timothy NJ Bullock, Alban Gaultier

University of Virginia

Growing evidence suggests that inflammatory responses of T cells rely on metabolic adaptations, especially elevated glucose metabolism, for bioenergetic and biosynthetic support. While this has generated excitement about targeting metabolic pathways for inflammatory conditions, the potential side effects associated with systemic administration of glycolysis inhibitors has tempered the translational potential of this concept. An alternative approach, and the premise of this work, is that machinery specifically upregulated to facilitate metabolic adaptation, and thus inflammation, may represent valuable targets by which inflammatory responses could be selectively defused. One candidate for this approach is Pyruvate kinase isoform M2 (PKM2), an isoform of the pyruvate kinase enzyme that is weakly expressed under homeostatic conditions and robustly upregulated by activated immune cells. While commonly known as an enzyme in the glycolytic cascade, PKM2 can double as a transcription factor. Indeed, it has been shown in myeloid cells that PKM2 can translocate to the nucleus, where it is serves as an essential co-activator of inflammatory gene signatures. PKM2 is a superb example of how machinery induced during metabolic adaptation can drive downstream inflammation. My goal is to identify how metabolic adaptations, like elevated PKM2, support pathologic immune cells during relapsing remitting multiple sclerosis (RRMS), a disease driven by inflammatory responses in the central nervous system. Using experimental autoimmune encephalomyelitis, a mouse model of RRMS, I have found that immune cells of the demyelinating CNS upregulate glycolytic machinery, including PKM2, and that levels of glycolytic machinery expression correlate with expression of inflammatory genes. Blocking PKM2 nuclear translocation with the drug TEPP-46 inhibits inflammatory responses of a diverse array of immune cells isolated from the demyelinating CNS at peak disease severity. In addition, I found that TEPP-46 selectively blocks the differentiation of IL-17A-producing T cells, a T cell subset known to drive autoinflammatory responses including, but not limited to RRMS. Taken together, these data reveal PKM2 is a potent modulator of CNS inflammation during immune-mediated demyelination.

Presenter: Shah, Masaud

Structural dynamics of opioid-bound MD2 and the mechanisms of subsequent TLR4 modulation

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The negative effect of opioids in analgesia and the initiation of central immune system, following TLR4 activation, have highlighted the underlying consequences for opioids pharmacodynamics. Successive studies have extended these findings and suggested that morphine and its opioid-receptorsâ€™ inactive metabolite M3G activate TLR4 pathway. However, detail structural insights of these ligands with TLR4/MD2 or MD2 alone have not yet been investigated. Here, we utilized extensive computational tools and highlighted the structural dynamics of the opioid-bound TLR4/MD2 and the underlying possible mechanism of their non-sterioselective activation of TLR4 pathway. Our docking results are supporting previous findings and further extend that the stability of morphine and naloxone, but not M3G, in MD2 cavity is TLR4 dependent. Further detail dynamics analysis suggests that morphine binds to the soluble MD2, but most likely this binding is reversible, which gains stability upon interacting with TLR4. Morphine establishes its interaction close to the Phe126 loop and facilitates the agonistic conformation of MD2C. M3G also confers MD2C and suggests the investment of H1 loop, in addition to Phe126 loop, in the ligand binding affinity and the overall stability of the TLR4/MD2-M3G complex. Naloxone remarkably switches the gating loop conformation from active (MD2C) to inactive (MD2O) form in MD2 and needs TLR4 for stabilization. Cumulatively, our findings suggest that ligand binding and receptor clustering occur subsequently in opioids-induced TLR4 signaling, and plasticity and pocket hydrophobicity in MD2 play key role in ligand recognition and accommodation.

Presenter: Shapleigh, Barbara

Determining the regulatory functions of lincRNA-Cox2 in vivo

Barbara Shapleigh, Roland Elling, John Rinn, Kate Fitzgerald and Susan Carpenter
University of California, Santa Cruz

Determining the regulatory functions of lincRNA-Cox2 in vivo Roland Elling1, Barbara Robinson Shapleigh2, John Rinn3, Kate Fitzgerald1 and Susan Carpenter2 1. Program in Innate Immunity, Division of Infectious Diseases, University of Massachusetts Medical School. 2. Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz. 3. Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA. Abstract Less than 3% of the human genome codes for protein, yet over 85% is actively transcribed. Why so much RNA is produced and whether it is all biologically relevant remains a fundamental unsolved problem. Long noncoding RNAs (lncRNAs), defined as transcripts greater than 200 nucleotides that do not encode proteins, represent the largest group of genes transcribed from the genome. LncRNAs have emerged as key regulators of biological processes, including genomic imprinting, development, and immunity. Notably, single lncRNAs can exert multiple functions within a cell. Some function locally (in cis) on neighboring genes, while others exert their effects at a distance (in trans) through formation of complexes with proteins or regions of DNA. Recent data suggest that differences in how lncRNAs are transcribed and spliced can impact how they function. Some lncRNA loci harbor enhancer elements, adding more complexity to their large repertoire of responses. Only 1% of lncRNAs have been characterized to date. Therefore, their functions remain poorly understood. We have identified lincRNA-Cox2 as a critical regulator of innate immune genes. LincRNA-Cox2 is highly inducible following inflammatory stimulation and it functions to represses expression of interferon-stimulated gene (ISG), yet is required for the expression of inflammatory genes, such as IL6. Our published data to date was carried out using in vitro cell culture systems. Here we describe our newly developed mouse models of lincRNA-Cox2 that aim to investigate the role that this gene plays in controlling innate immune responses in vivo. We have generated a lincRNA-Cox2 knockout mouse in which the entire transcript has been removed and replaced with a LacZ cassette using homologous recombination. We have also generated a lincRNA-Cox2 splicing mutant mouse in which we have removed the major intron of its transcript using Cas9/CRISPR technology. These mouse models will enable us to determine how this gene functions and whether it is critical to control host responses to infections in vivo.

Presenter: Shin, Hyeon-Jun

Evaluation of TLR4 inflammatory pathway using TIRAP-derived peptides

Hyeon-Jun Shin, Xiangai Gui and Sangdun Choi

Department of Molecular Science and Technology, Ajou University, Suwon, Korea

Activation of Toll-like receptor 4 (TLR4) ensues the increased secretion of inflammatory cytokines, reactive oxygen species and NO production. TLR4-induced exaggerated immune responses are associated with the initiation of various diseases as well as aggravating the inflammatory diseases including rheumatoid arthritis, sepsis and acute lung injury. TIRAP (Toll/interleukin-1 receptor domain containing adaptor protein) is an important adaptor molecule that is involved in TLR4 signaling propagation. Its activation leads to the subsequent activation of NF-κB and mitogen activated protein kinases (MAPKs; extracellular regulated kinase, c-Jun N-terminal Kinase and p38), which then results in cytokine secretion and inflammatory responses. Herein, TIRAP-derived peptide has been evaluated to block TLR4 pathway. This peptide inhibited TLR4-mediated activation of NF-κB, MAPKs and other cytokines. The peptide hindered the induction of iNOS, COX2, NO and ROS. The peptide also diminished systemic cytokine responses elicited in vivo by lipopolysaccharide challenge. This peptide represents a TLR4 pathway inhibitor that blocks protein-protein interaction between Toll/interleukin 1 receptor domains and, thus, TLR4-mediated immune responses.

Presenter: Smith, Drake

Propagating Humanized BLT Mice for the Study of Human Immunology and Immunotherapy

Drake Smith, Levina J. Lin, Heesung Moon, Alexander T. Pham, Xi Wang, Siyuan Liu, Sunjong Ji, Valerie Rezek, Saki Shimizu, Marlene Ruiz, Jennifer Lam, Deanna M. Janzen, Sanaz Memarzadeh, Donald B. Kohn, Jerome A. Zack, Scott G. Kitchen, Dong Sung An, Lili Yang

UCLA

The humanized bone marrow-liver-thymus (BLT) mouse model harbors a nearly complete human immune system, therefore providing a powerful tool to study human immunology and immunotherapy. However, its application is greatly limited by the restricted supply of human CD34+ hematopoietic stem cells and fetal thymus tissues that are needed to generate these mice. The restriction is especially significant for the study of human immune systems with special genetic traits, such as certain human leukocyte antigen (HLA) haplotypes or monogene deficiencies. To circumvent this critical limitation, we have developed a method to quickly propagate established BLT mice. Through secondary transfer of bone marrow cells and human thymus implants from BLT mice into NSG (NOD/SCID/IL-2R?-/-) recipient mice, we were able to expand one primary BLT mouse into a colony of 4-5 proBLT (propagated BLT) mice in 6-8 weeks. These proBLT mice reconstituted human immune cells, including T cells, at levels comparable to those of their primary BLT donor mouse. They also faithfully inherited the human immune cell genetic traits from their donor BLT mouse, such as the HLA-A2 haplotype that is of special interest for studying HLA-A2-restricted human T cell immunotherapies. Moreover, an EGFP reporter gene engineered into the human immune system was stably passed from BLT to proBLT mice, making proBLT mice suitable for studying human immune cell gene therapy. This method provides an opportunity to overcome a critical hurdle to utilizing the BLT humanized mouse model and enables its more widespread use as a valuable preclinical research tool.

Presenter: Smith, Norah L.

The fate of CD8+ T cells during infection is linked to their developmental origin

Norah L. Smith, Arnold Reynaldi, Jocelyn Wang, Neva Watson, Kito Nzingha, Kristel YeeMon, Seth Peng, Jennifer Grenier, Andrew Grimson, Miles P. Davenport, Brian D. Rudd

Cornell University

Intracellular pathogens drive a CD8+ T cell response typified by robust clonal expansion and phenotypic diversification into multiple subsets of effector cells. For example, following infection, some cells display markers for terminal differentiation (KLRG1), are efficient at clearing infected cells but are short-lived while others upregulate cytokine receptors (IL-7R), important for long term cell survival and the establishment of immunological memory. While we know that these subsets of effector cells exist, the mechanisms that regulate the fate of naive CD8+ T cells after microbial challenge remain poorly understood. Previous studies suggest heterogeneity in the effector pool is driven by differences in TCR avidity, asymmetric cell division, and environmental cues. However, although the pool of naive CD8+ T cells is comprised of clonotypes that were produced during various stages of life, one variable that has not been closely examined is developmental origin. In fetal life, the first wave of hematopoietic stem cells (HSCs) to colonize the thymus come from fetal liver precursors. Later in life, HSCs come from the bone marrow. To determine whether the developmental origin of naive CD8+ T cells plays a deterministic role in their fate after infection, we developed a system to â€˜timestampâ€™ CD8+ T cells from various stages of development (1 day, 7 days, and 28 days) and examined their phenotype and behavior in 8-week-old adult mice. We found naive CD8+ T cells made early in life express higher starting levels of effector molecules (T-bet) and predominantly display a CD44hi CD122hi memory phenotype whereas cells made later in life are CD44lo CD122lo. RNA sequencing reveals that cells from differing developmental origins have unique transcriptional profiles. In vitro stimulation of stamped populations shows that fetal- and neonatal-derived CD8+ T cells are hypersensitive to IL12/IL18, proliferate and make effector molecules more rapidly than cells made in older animals. In response to infection in adulthood, both polyclonal and monoclonal CD8+ T cells produced in early life expand rapidly and skew towards a short-lived effector phenotype. These data indicate that the spectrum of CD8+ T cell differentiation observed after infection is influenced by when the responding cells were initially made.

Presenter: So Jin, Bing

GM-CSF plays a pathogenic role in autoimmunity to the neuroretina in the absence of Th1 and Th17 lineage cytokines

Bing So Jin, Phyllis B. Silver, Reiko Horai, Yingyos Jittayasothorn, Rachel R. Caspi

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Autoimmune uveitis is a complex group of sight-threatening diseases caused by activated retina-specific Th1 or Th17 cells that acquired the ability to cross the blood-retinal barrier, but their respective contribution to autoimmune uveitis is not fully understood. In this study, we used mice deficient in IL-17A or IFN-? (GKO), or both IL-17 and IFN-? (DKO) to dissect the role of Th1 and Th17 lineage-specific cytokines in pathogenesis of uveitis. Experimental autoimmune uveitis (EAU) was induced by active immunization with the retinal autoantigen IRBP in complete Freunds adjuvant. IL-17A-/- mice were protected from EAU, whereas GKO mice had exacerbated disease. Surprisingly, DKO mice were fully susceptible to EAU, with scores similar to WT controls. To test whether other lineage-specific proinflammatory cytokines compensate for lack of IL-17A and IFN-?, EAU-challenged DKO mice were treated with blocking antibodies to IL-17F, IL-22, TNF-a, or GM-CSF. Blockade of IL-17F, IL-22, or TNF-a in DKO mice did not affect the severity of EAU. By contrast, treatment of DKO mice with anti-GM-CSF antibody, either as prevention or as a reversal paradigm, significantly suppressed EAU. These results suggest that IL-17F, IL-22 or TNF-a individually are dispensable, but GM-CSF appears to play a major and nonredundant role in development of EAU when IFN-? and IL-17A are both absent. To examine whether GM-CSF-secreting T cells (ThGM) are pathogenic effectors, IRBP-specific T cells (from R161H mice) were polarized in vitro to ThGM, Th1 or Th17 phenotypes, and were adoptively transferred to naive WT mice. A high proportion of ThGM-polarized cells produced GM-CSF, and many co-produced IFN-?, Notably, these cells induced a more severe disease than did Th17 cells, (but less severe than Th1 cells), supporting the notion that ThGM cells are also uveitogenic. Together, these results indicate that â€˜classicâ€™ Th1 and Th17 cells are not the only pathogenic effectors in uveitis, and that other inflammatory cytokine(s), such as GM-CSF, can also drive disease.

Presenter: Sullivan, Jenna

T cell specific dysregulation of Foxo1 results in spontaneous inflammatory disease

Jenna Sullivan, Kerri Thomas, Jeffrey Duggan 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. In conventional T cells, Foxo1 is phosphorylated downstream of the PI3K/Akt/mTOR signaling pathway resulting in its inactivation and translocation from the nucleus and subsequent altered transcription of downstream targets. While many studies have examined the effects of Foxo1 deficiency, few have addressed the role of proper downregulation of Foxo1. To address this question we have obtained mice that carry a non-phosphorylatable Foxo1 allele (Foxo1CA) knocked into the ROSA26 locus, along with a â€˜stop-floxâ€™ cassette that prevents expression. We have crossed Foxo1CA mice to CD4-Cre mice (CD4cre Foxo1CA), resulting in deletion of the â€˜stop-floxâ€™ cassette and constitutive activation of Foxo1 in both CD4 and CD8 T cells beginning at the double positive stage of thymic development. Interestingly neonate thymi contain normal numbers of double positive thymocytes by have reduced mature single positive CD4 T cells with decreased cortex area and increased cell death. Mice which are unable to properly regulate Foxo1 in T cells are characterized grossly by runting, dermatitis and splenomegaly. In the periphery CD4cre Foxo1CA mice have reduced CD4 T cell populations comprised mainly of activated effector T cells and a dramatically reduced Treg population. Together this data demonstrates that proper regulation of Foxo1 is essential for thymic development and prevention of autoimmunity.

Presenter: Tanaka, Shigeru

The role of KAP1 in regulatory T cells

Shigeru Tanaka, Steven F Ziegler
Benaroya Research Institute

Not to be placed on website

Presenter: Tellez Freitas, Claudia M

Naive Helper T Cells with high CD5 expression have increased calcium signaling

Claudia M Tellez Freitas, Garrett J Hamblin; Carlee M Larsen; Scott Weber

Brigham Young University

The adaptive immune response is orchestrated by T helper cells and interactions between the T cell receptor (TCR), peptide MHC (pMHC) and co-receptors. These TCR-pMHC interactions initiate calcium signaling cascades which determine T cell activation and function. CD5 is a co-receptor that plays an important role in regulating T cell signaling and fate during thymocyte education. CD5 surface expression on mature single positive thymocytes correlates with the TCR signal strength for positive selecting self-ligands. CD5 also plays a role in T cell function after thymic development is complete. Peripheral T cells with higher CD5 expression respond better to foreign antigen than those with lower CD5 expression and are CD5 high T cells are enriched in memory populations. In our study, we examined the role of CD5 expression and calcium mobilization in the primary response of T cells using two Listeria monocytogenes specific T helper cells (LLO118 and LLO56). These T cells recognize the same immunodominant epitope (LLO190-205) of L. monocytogenes and have divergent primary and secondary responses as well as different levels of CD5 expression. We characterized the role of CD5 expression and calcium influx in these CD5 high and CD5 low T cells over the course of 8 days. We found significantly different calcium signaling levels in naive and day 3 post-stimulation LLO56 and LLO118 T helper cells. To further investigate the role CD5 expression plays in calcium mobilization, we measured the calcium influx in T cells from LLO118-CD5 knockout mice versus those from LLO56-CD5 knockout mice. We found that CD5 expression is important in regulating calcium mobilization in the CD5 high naive LLO56 T cells during the initial response to antigen and as CD5 levels decrease over time its role in regulating calcium also decreases.

Presenter: Tenthorey, Jeannette

Recognition of multiple motifs on bacterial ligands by NAIP receptors constrains bacterial immune evasion

Jeannette Tenthorey, Ella Hartenian, Elise Adamson, Russell Vance

University of California, Berkeley

The innate immune system faces the challenging task of using a small number of receptors to recognize diverse classes of microbes based on features that distinguish microbes from host cells. Because microbes can rapidly evolve to evade detection, it might be beneficial for innate immune receptors to bind promiscuously to variable microbial ligands. On the other hand, promiscuous binding might also allow for aberrant recognition of self ligands to trigger auto-immune responses. We investigated how the innate immune system balances the specificity of ligand recognition with robustness to mutagenesis in the context of NAIP inflammasomes. Mouse NAIP paralogs bind to conserved bacterial proteins, including flagellin and structural components of the type III secretion system, that are translocated by the pathogen into the host cell cytosol. To determine how NAIPs recognize their target ligands, we screened a library of mutants in two of these ligands for their ability to activate their cognate NAIPs. In each case, we found that NAIP activation requires at least two ligand motifs that are segregated in primary sequence. Furthermore, both motifs bound to their cognate NAIP, suggesting that each is directly recognized. Importantly, single point mutations in either recognition motif are not sufficient to evade NAIP recognition. Instead, both sites must be mutated to evade immune recognition. Mutants that escape NAIP recognition are correspondingly more likely to be non-functional. We suggest that a bipartite mode of ligand recognition is a generalizable strategy for constraining bacterial immune evasion while simultaneously increasing the stringency of off-target recognition.

Presenter: Tomala, Jakub

Immunogenic potential of high hydrostatic pressure-treated cancer cells

Jakub Tomala, Iva Truxova1,2, Abhishek D. Garg4, Jakub Tomala5, Patrizia Agostinis4, Pierre Francois Cartron3, Sarka Vosahlikova1, Marek Kovar5, Radek Spisek1,2 and Jitka Fucikova1,2

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Immunogenic cell death (ICD) is an immunogenic form of apoptosis caused by certain chemotherapeutics (e.g. anthracyclines, mitoxantrone, oxaliplatin or bortezomib), ionizing irradiation, oncolytic viruses and Hyperticin-based photodynamic therapy. It is mediated largely by spatiotemporally-defined release or exposure of danger signals or damage-associated molecular patterns (DAMPs) that can function as either adjuvants or danger signals for the innate immune system leading to the induction of host protective anticancer immunity. Intracellular chaperone calreticulin represents one of the major molecules included in ICD. Recently, novel method of ICD induction, high hydrostatic pressure (HHP), was found, promoting key characteristics of ICD in similar way as immunogenic chemotherapy and ionizing irradiation. Here, we demonstrate that cancer cells succumbing to HHP induce CD4+ and CD8+ T cell-dependent protective immunity in vivo. Moreover, we show that cell death induction by HHP relies on the overproduction of reactive oxygen species (ROS), causing rapid establishment of the integrated stress response, eIF2α phosphorylation by PERK, and sequential caspase-2, -8 and -3 activation. Thus, caspase-2 mediates key functions in the interaction between dying cancer cells and antigen presenting cells. Our results indicate that the ROS->PERK->eIF2α->caspase-2 signaling pathway is central for the perception of HHP-driven cell death as immunogenic. Acknowledgement: This work was supported by SOTIO research contract (HS0045), LQ1604 NPU II provided by MEYS, CZ.1.O5/1.1.00/02.0109 BIOCEV provided by ERDF and MEYS and Institutional Research Concept RVO 61388971.

Presenter: Townsend, Michelle

Is Interleukin-10 a key factor in colon cancer metastasis?

Michelle Townsend, Abi Felsted, Edwin Velazquez, Evita Weagel, Richard Robison, and Kim Oâ€™Neill
Brigham Young University

Interleukin 10 (IL-10) is a crucial cytokine synthesis inhibitory factor involved in cancer development. As a potent anti-inflammatory molecule, IL-10 is commonly elevated within the tumor microenvironment. IL-10 is primarily produced by helper T-cells along with regulatory T-cells and functions by inhibiting Th1-mediated responses. We examined the abundance of IL-10 expression in patients with colon adenocarcinoma and lymph node metastatic adenocarcinoma from the colon in order to evaluate whether IL-10 expression was increased within metastatic cancers. Tissue from 100 patients ranging in age from 30 to 79 with either colon adenocarcinoma, lymph node metastatic adenocarcinoma (from the colon), adenoma, normal adjacent tissue, and normal colon tissue were obtained and stained for IL-10 expression using standard Immunohistochemistry techniques. Briefly, tissues were treated with a monoclonal anti-IL-10 antibody along with a GADPH positive control and an isotype negative control. Tissues were incubated with an HRP-polymer conjugated antibody, followed by a DAB substrate which reacts with antibody-antigen binding sites to produce a colorimetric change within the tissue. Tissues were further quantified using Image J software and an IHC toolbox plugin in order to measure DAB staining intensity within samples and data is reported in gray value. We found a significant difference between the levels of IL-10 between metastatic tissue and malignant adenocarcinoma. While only 23% of the Adenocarcinoma samples showed a positive level of IL-10 binding, over 55% of the metastatic samples had significant IL-10 expression. These data indicate a significant elevation in IL-10 expression in tumor tissue derived from a metastatic site. In addition, we also found differences in IL-10 presence between patients. While some patient tissue showed a significant up-regulation of IL-10 ($p = 0.0018$), other patients had no apparent up-regulation in comparison to normal controls. This indicates that IL-10 production is variable between patients and may have implications on recurrence and outcome. This study shows that the production of IL-10 from tumor cells is not only variable between patients, but is also increased within metastatic tumor tissue.

Presenter: Tuladhar, Shraddha

Determining how Toxoplasma strains drive strain-specific CNS immune response

Shraddha Tuladhar, Yarah Ghotmi, Apoorva Bhaskara, Joseph S. Lagas, and Anita Koshy

Department of Immunobiology, Bio5 Institute, University of Arizona

Toxoplasma gondii is an obligate intracellular parasite that infects up to 1/3 of the worldâ€™s population. Though this persistent infection is asymptomatic in most, in immunocompromised individuals, this infection can give rise to symptoms ranging from fever, focal neuro-inflammation to death. While the determinants of disease variability are poorly understood, recent human data suggest that the genotype of the infecting Toxoplasma strain may influence disease outcomes. Additionally, prior work in the mouse model has shown that different Toxoplasma strains provoke different central nervous system (CNS) immune responses and recent in vivo data have revealed that strain-specific polymorphic effector proteins injected into host cells can lead to different innate immune responses. Thus, we hypothesize that different Toxoplasma strains provoke distinct, strain-specific immune responses, which in turn would affect disease severity. To test this hypothesis, we used quantitative immunohistochemistry to compare CNS immune responses in mice infected with either type II or type III parasites. At 3 weeks post infection (wpi), we found that despite having similar parasite burden, the CNS of type III-infected mice had a more pro-inflammatory CNS immune response (macrophages and T-cell response, and cytokines/chemokines) as compared to type II-infected mice. Consistent with these findings, our flow cytometry analysis of immune cells isolated from the spleen and brain of 3 wpi mice showed that type III-infected mice had a significantly lower numbers of alternatively activated macrophages (AAMs) and regulatory T cells as compared to type II-infected mice. However, flow cytometry analysis on splenocytes during acute infection (0.5 and 1.5 wpi), showed that type III-infected mice had higher numbers of AAMs. Taking these data together, we propose that by inducing a robust pro-inflammatory response early on, type II-infection provokes a compensatory anti-inflammatory response that will ultimately hinder the host from eliminating CNS parasites. Conversely, early in infection type III parasites elicit a less inflammatory response, allowing for a subtle increase in the peripheral parasite burden that then drives a more pro-inflammatory response, resulting in an immune response that will effectively clear CNS parasites. This model predicts that despite having equivalent parasite burdens early in CNS infection, type III-infected mice should have a lower CNS parasite burden chronically. We are currently testing this possibility by evaluating the CNS parasite burden at 16 wpi. We also hypothesize that polymorphic effector proteins drive the differences in the initial immune response. In particular, we hypothesize that ROP16 and GRA15, two effector proteins that differentially modulate major immune signaling pathways in a Toxoplasma strain-specific manner, underlie these immune response differences. Currently we are engineering parasites to test this hypothesis.

Presenter: Uhrlaub, Jennifer L.

Role of immune evasion mechanisms in priming and maintaining CD8 T cell responses to poxvirus infection across lifespan

Jennifer L. Uhrlaub, Megan J. Smithey, Janko Nikolich-Zugich

University of Arizona, Department of Immunobiology

Cowpox virus (CPXV) employs two proteins that effectively prevent the expression of MHC Class I on the surface of infected cells: CPXV012 and CPXV203. These immune evasion proteins reduce the capacity of the host immune system to detect infected cells, although the extent of their influence on the adaptive immune response remains incompletely understood. We found that with regard to CD8 T cell responses, this influence was strongly age-dependent. In young mice, CD8 T cell responses to CPXV, presumed to be the result of cross-presentation, remained fully functional and without any obvious immune response defects. In aging animals, however, CD8 T cell responses to CPXV were diminished, with low functional capacity. We provide evidence that restoration of direct Ag presentation through the deletion of CPXV012 and CPXV203 elicited a more robust primary CD8 T cell response with superior functional capacity during recall. This mechanism for improved CD8 T cell function was conserved across life-span. We discuss relevance of this manipulation with regard to improved vaccine efficacy, particularly in old animals.

Presenter: Utzschneider, Daniel

The T cell memory phenotype is actively maintained by the expression of FOXO1

Daniel Utzschneider, Stephen Hedrick

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Functional immune memory governed by CD8 T cells is indispensable for protection against bacterial and viral re-infection. In order to provide such long-term protection, memory T cells must survive over longer periods in the absence of antigenic stimulation by undergoing homeostatic self-renewal. By using a Tamoxifen-dependent Cre recombinase to deplete Foxo1 in differentiated memory CD8 T cells, we delineate the role of the transcription factor in sustaining a memory population. Interestingly, the sudden depletion of Foxo1 immediately impacted multiple functional and molecular properties of a memory population such as expression of IL7Ra, CD62L, Tcf1, and Eomes. In line with the deterioration of these molecules, we observed a gradual contraction of the memory population over time. This impaired maintenance derived from a reduced number of cells undergoing homeostatic proliferation and, more strikingly, these fewer proliferating cells were unable to regenerate themselves in the absence of FOXO1 and instead gave rise to progeny lacking hallmarks of conventional memory T cells. Despite this inability to renew, FOXO1-depleted memory cells sustained the ability to mount a robust recall response following antigen re-challenge â€“ irrespective if they were challenged immediately after the FOXO1-depletion or following a longer resting period. The resulting FOXO1-deficient cells diminished in number at an accelerated rate compared with WT T cells. We show that the functional and molecular properties of a memory T cell population and thus its ability to be sustained long-term are dependent on the perduring expression of the transcription factor FOXO1. Moreover, we illustrate that resting memory T cells exhibit continuous transcriptional plasticity.

Presenter: Vaden, Kiara

Determining the Optimal TCR:pepMHC Affinity for CD4+ T cell Primary and Memory Response?

Kiara Vaden, John C. Hancock III, K. Scott Weber

BYU

The strength of TCR:pepMHC interaction (affinity) helps determine T cell activation and differentiation, which influences the outcome of the immune response to infection. A predominant hypothesis has been that stronger TCR:pepMHC affinity produces better T cell activation and proliferation than weaker interactions. Recent research, however, suggests that low or intermediate affinity may promote strong T cell responses. This elicits the need to determine the affinity conditions necessary for the best CD4+ T cell responses. We have used two T cells from transgenic Rag1 -/- mouse lines, called LLO118 and LLO56, to better understand this question. The TCRs on these CD4+ T cells recognize the same epitope from Listeria monocytogenes (listeriolysin O (LLO190-205), however, they differ by fifteen amino acids and respond differently in vivo. Using three L. monocytogenes APL mutants, we have studied LLO118 and LLO56 T cell response in vitro and in vivo by measuring their proliferation upon exposure to the epitope. We have found that for LLO118, the mutants that act as partial agonists in vitro generate the strongest primary response in vivo. Understanding how TCR:pepMHC affinity affects T cell function is crucial for the development of cancer immunotherapies, vaccines, and understanding immune-mediated diseases such as autoimmunity and graft rejection.

Presenter: Wang, Jocelyn (Jie)

Lin28b alters the neonatal CD8+ T cell response to infection by reprogramming cellular metabolism

Jocelyn (Jie) Wang, Erin Wissink, Neva Waston, Norah L. Smith, Andrew Grimson, and Brian D. Rudd
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Neonates often generate incomplete immunity against intracellular pathogens. As CD8+ T cells are essential for clearing intracellular pathogens, it is crucial to understand why they behave differently than adults during infection. We previously showed that neonatal CD8+ T cells fail to form memory because they rapidly differentiate into short-lived effectors. However, the underlying basis for these age-related differences is unclear. To gain insight into these age-related differences, we first examined gene expression profiles in CD8+ T cells isolated from the thymus and periphery of neonatal and adult mice. Interestingly, we observed distinct transcriptomes in neonatal CD8+ T cells from the time they were initially created in the thymus, indicating that neonatal CD8+ T cells are made differently than their adult counterparts. To examine whether different hematopoietic stem cells (fetal liver vs. adult bone marrow) underlie these age-related differences, we performed intrathymic transfers with fetal and adult progenitors and compared how their progeny responded to infection with listeria monocytogenes. Whereas fetal-derived CD8+ T cells selectively formed short-lived effectors, the adult-derived cells preferentially gave rise to memory precursors. Given that Lin28b has been shown to play an important role in fetal lymphopoiesis and is selectively expressed in neonatal CD8+ T cells, we next asked whether ectopic expression of Lin28b in adult CD8+ T cells enables them to behave more like neonatal CD8+ T cells. Strikingly, adult CD8+ T cells with overexpressed Lin28b showed higher OXPHOS and glycolytic activities upon activation and preferentially formed short-lived effector cells compared to wild-type cells. However, when mTOR activity was limited with rapamycin, the defective formation of Lin28b-induced memory precursor cells could be restored. This data is consistent with the metabolic and phenotypic differences observed between adult and neonatal CD8+ T cells. Collectively, our data suggests that Lin28b regulates the neonatal CD8+ T cell response through the reprogramming of cellular metabolism.

Presenter: Wang, Ziming

Bortezomib sensitizes cancer stem cells from solid human tumors to natural killer cell-mediated killing

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Cancer stem cells (CSCs) from solid and hematopoietic tumors resist conventional cytotoxic therapies that target rapidly proliferating cells. Thus, residual CSCs can hide within the tumor niche and seed relapse and metastasis. Due to their relapse potential there is an urgent need to identify ways to therapeutically target CSCs. We previously found that cells expressing high amounts of the stem cell associated protein aldehyde dehydrogenase (ALDH) are effectively killed by activated natural killer (NK) lymphocytes. NK cells are known to kill malignant cells though apoptotic processes inherent to the target cell, such as TRAIL-DR5 or Fas-FasL binding, without prior immunization. We and others have also found that the FDA approved proteasome inhibitor, bortezomib, sensitizes tumor cells to NK cell killing by upregulating DR5 and intracellular machinery associated with apoptosis. Based on these, we investigated the effects of bortezomib to promote NK cell killing of ALDHbright CSCs. We evaluated CSCs derived from glioblastoma (GBM), in vitro and in vivo, for the induction of receptors associated with NK cell mediated killing, and for their susceptibility to NK killing after treatment. In this study, bortezomib led to a 3-fold increase in the percentage of viable ALDHbright GBM, in vitro, compared to untreated controls. Moreover, it enhanced the median fluoresence intensity (MFI) of Fas, DR5, and MICA/B in either U87 and or T98G cell lines, on both ALDHbright and ALDHdim cells. Bortezomib had also increased the percentage of viable ALDHbright in patient-derived primary GBM in vitro compared to untreated controls. Additionally, the pretreatment of bortezomib leads to a 98% decrease in viable ALDHbright cells following NK cytotoxicity assays in vitro. In vivo, it improved the efficacy of adoptive NK cell therapy in GBM xenograft models. These data indicate that the combined use of bortezomib with activated natural killer cells could act as a potential anti-CSC strategy to improve outcomes for patients with GBM.

Presenter: Webb, Lindsay

Epigenetic modifications modulate CD4+ T cell responses and autoimmunity

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In the autoimmune disease Multiple Sclerosis (MS), pro-inflammatory T helper (Th) 1 and Th17 cell responses overwhelm regulatory Th2 and Treg responses. MS treatments that suppress inflammatory T cell responses and promote regulatory T cell responses would be beneficial to the field. Epigenetic modifications are essential to T cell expansion and effector functions. Interestingly, it is known that pan-methylation reaction inhibitors suppress T cell proliferation and experimental autoimmune encephalomyelitis (EAE), but the enzyme responsible for these effects remains to be determined. Protein arginine methyltransferase 5 (PRMT5), the major Type II methyltransferase enzyme, mediates symmetric dimethylation of arginine residues of histones. Although PRMT5 has been shown to be up-regulated in many lymphoid malignancies, currently there is no known role for PRMT5 in T cells. In this study, we use memory Th cells and animal models to study the role of PRMT5 in T cell responses and autoimmunity. PRMT5 was up-regulated in memory Th1 and Th2 cells upon activation and treatment with novel PRMT5-selective inhibitors differentially impacted pathogenic Th1 and benign Th2 cell proliferation. Finally, we show that PRMT5-selective inhibitors ameliorate disease severity in EAE, the mouse model of MS. These results indicate that PRMT5 may be a promising therapeutic target in T cell-mediated autoimmune diseases such as Multiple Sclerosis.

Presenter: Wong, Rachel

B cell receptor affinity may instruct the long-lived plasma cell fate

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Germinal center B cells are selected to enter either the memory B cell or the long-lived plasma cell compartment, yet the underlying mechanisms that control this decision are unknown. Our recent work utilizing a West Nile Virus (WNV) infection system demonstrated that the antigen specificity between the memory B cell and long-lived plasma cell compartments differed. Long-lived plasma cells primarily recognized the dominantly neutralizing epitope, the lateral ridge, of West Nile Virus envelope protein domain III (DIII). In contrast, memory B cells could recognize the lateral ridge epitope and non-lateral ridge epitopes. We hypothesized that non-lateral ridge-specific B cell receptors fail to reach an affinity threshold that promotes the long-lived plasma cell fate. To assess this, we utilized DIII-tetramers that are or are not mutated at the lateral ridge to identify antigen-specific memory B cells and long-lived plasma cells. V(D)J sequences from these cells were cloned into expression vectors for antibody production. The binding patterns of these monoclonal antibodies, at the affinity matured and germline-reverted states, were assessed by ELISA and Bio-Layer Interferometry (BLI). Preliminary data indicates that non-lateral ridge-specific antibodies bind DIII more poorly than lateral ridge-specific antibodies. This difference is more striking when germline forms of these antibodies are compared. Non-lateral ridge-specific antibodies are present during early germinal center reactions when memory B cells are formed, but are progressively lost at later timepoints when long-lived plasma cells emerge. Our preliminary data suggest the existence of an antibody affinity threshold that promotes germinal center B cell retention and long-lived plasma cell selection.

Presenter: Yamane, Hidehiro

E-box binding protein HEB promotes the maturation into germinal center TFH cells by preventing re-expression of EBI2 on pre-TFH cells through the suppression of Eomes

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NIAID, NIH

E-box binding proteins (E-proteins) have recently been demonstrated to regulate follicular B helper T (TFH) cell differentiation, but the underlying mechanisms remain elusive. To clarify the molecular basis of E-protein-mediated TFH differentiation, we generated mouse strains with a peripheral T cell-specific deletion of either E2A (E2AcKO) or HEB (HEBcKO) or both (E2A/HEBcKO) on an OT-II TCR-transgenic background. Naive CD4+ OT-II T cells from these mutant mice were adoptively transferred into C57BL/6 mice, and the recipients were immunized with OVA/alum on the following day of the adoptive transfer. Seven days after immunization, E2A/HEBcKO donor cells in the spleen had a substantial defect in germinal center (GC) TFH differentiation with a reduction in clonal expansion by 2 to 3-fold. E2AcKO donor cells differentiated into GC TFH cells comparably to WT counterparts. By contrast, HEBcKO donor cells underwent pre-TFH cell differentiation but failed to maturate into GC TFH cells with normal clonal expansion, implying the importance of HEB, but not E2A, in GC TFH differentiation. Gene expression profiling analysis revealed that 5 days after immunization, HEBcKO pre-TFH cells had no defect in the expression of signature Tfh-related genes but a substantial increase in the expression of the Gpr183 gene encoding Epstein-Barr virus-induced G-protein coupled receptor 2 (EBI2), a receptor for 7α,25-dihydroxycholesterol secreted by stromal cells residing in the outer T cell zone. Surface expression of EBI2 on HEBcKO donor cells was induced normally at day 2 and downregulated at day 4 post-immunization comparably to that on WT donor cells. However, at day 5 post-immunization or later, HEBcKO pre-TFH cells re-expressed high levels of EBI2, whereas WT counterparts kept downregulating it. Consistent with the data on the surface expression of EBI2, HEBcKO donor cells accumulated in the T/B border and little or no such cells were found in either B cell follicles or GCs at day 7 post-immunization. The impaired GC TFH differentiation by HEB deficiency was accompanied by transient and aberrant expression of Eomes during the first 2 days of priming. Of note, further conditional loss of Eomes prevented HEBcKO donor cells from re-expressing EBI2 and rescued them from the GC TFH deficit. In conclusion, HEB does not directly regulate the expression of the signature TFH-related genes but fine-tunes the localization of pre-TFH cells by preventing EBI2 re-expression, allowing for their entry to B cell follicles to undergo subsequent maturation into GC TFH cells. Moreover, the HEB-mediated suppression of Eomes expression during the early priming phase plays a critical role in rendering EBI2 suppressed on pre-TFH cells. This work was supported by the Intramural Research Program of NIAID, NIH.

Presenter: Yang, Lili

Stem Cell-Engineered Invariant Natural Killer T Cells for Cancer Therapy

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Invariant natural killer T (iNKT) cells comprise a small population of aÃŸ T lymphocytes. They bridge the innate and adaptive immune systems and mediate strong and rapid responses to many diseases, including cancer, infections, allergies and autoimmunity. However, the study of iNKT cell biology and the therapeutic applications of these cells are greatly limited by their small numbers in vivo (~0.01-1% in mouse and human blood). Here, we report a new method to generate large numbers of iNKT cells in mice through T cell receptor (TCR) gene engineering of hematopoietic stem cells (HSCs). We showed that iNKT TCR-engineered HSCs could generate a clonal population of iNKT cells. These HSC-engineered iNKT cells displayed the typical iNKT cell phenotype and functionality. They followed a two-stage developmental path, first in thymus and then in the periphery, resembling that of endogenous iNKT cells. When tested in a mouse melanoma lung metastasis model, the HSC-engineered iNKT cells effectively protected mice from tumor metastasis. This method provides a powerful and high-throughput tool to investigate the in vivo development and functionality of clonal iNKT cells in mice. More importantly, this method takes advantage of the self-renewal and longevity of HSCs to generate a long-term supply of engineered iNKT cells, thus opening up a new avenue for iNKT cell-based immunotherapy.

Presenter: Zaitseva, Galina

Differential effects of endosulfan on NK cells divided into the two classical phenotypes CD56dimCD16bright and CD56brightCD16dim

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INTRODUCTION. Endosulfan is a xeno-estrogen organochlorine pesticide. Bioaccumulation of past pesticide use, as well as environmental effects of current use indicate that the investigation of the effects of endosulfan exposure is of current importance. Endosulfan exposure has been strongly correlated with neural and endocrine system damage. The effects of this pesticide on the immune system are not fully characterized. We have previously shown the effects of endosulfan on non specific activation, proliferation, apoptosis and senescence in immune cells from Nile tilapia. The effects on cytotoxicity are not well characterized. In humans, the NK cell subset responsible for cytotoxic activity are the CD56dimCD16+/- cells, while CD56brightCD16dim cells have been characterized as cytokine producing and possible regulatory cells. In some species endosulfan has been reported to have No Observed Effect Level (NOEL) with respect to oncogenic or teratogenic effects below 6 ppm (15 ÂµM) exposure or 2kg/mg/day dosis. Based on previous work, we established a range of experimental exposure from 0.1 to 17 uM. Objective: To characterize the effects of endosulfan on NK and NK-like cells across a concentration range from very low to probable environmental levels. Methods: Flow cytometry was used to characterize the following cells exposed to endosulfan: NCC cells from Nile Tilapia fish, HL-60 targets, cell death measured by PI; NKL cell line with K562-GFP targets, cell death measured by 7-AAD; PBMCs, with and without PHA mitogen, from healthy volunteers. Proliferation was measured using WST-1; cytokines were quantified using the Bioplex Pro assay. CONCLUSION Endosulfan inhibits proliferation in lymphocytes and cytotoxic activity in fish NCC cells, and to a lesser extent, human NKL cells. NK cells show increased apoptosis with endosulfan exposure. NK cells from PBMC can be divided into two populations with distinct responses: The cytotoxic CD56dimCD16bright have an activated phenotype, but are decreased with endosulfan exposure. CD56bright cytokine producing or regulatory cells are relatively increased with endosulfan exposure. Cytokine production dramatically increases with endosulfan exposure, principally IFN-gamma.